UNIVERSITY OF ALBERTA
MAR 8 1977
THESES CANADIENNES SUR MICROFICHE

30683

National Library of Canada / Bibliothèque nationale du Canada — CANADIAN THESES ON MICROFICHE / THÈSES CANADIENNES SUR MICROFICHE

NAME OF AUTHOR/*NOM DE L'AUTEUR* PHILLIP F. GARDINER

TITLE OF THESIS/*TITRE DE LA THÈSE* METABOLIC FACTORS ASSOCIATED WITH TENSION DECLINE IN THE ELECTRICALLY STIMULATED IN SITU CANINE GASTROCNEMIUS MUSCLE ~~MUSCLE~~ - UNIVERSITY OF ALBERTA

UNIVERSITY/*UNIVERSITÉ*

DEGREE FOR WHICH THESIS WAS PRESENTED/*GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Ph.D.

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1976

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* DR. HOWARD A. WENGER

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/*DATÉ* Mar 9/76 SIGNED/*SIGNÉ* P. F. Gardiner

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ* Department of Kinesiology
University of California, Los Angeles
Los Angeles, California 90024

INFORMATION TO USERS

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

This copy was produced from a microfiche copy of the original document. The quality of the copy is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Canadian Theses Division
Cataloguing Branch
National Library of Canada
Ottawa, Canada K1A 0N4

AVIS AUX USAGERS

LA THESE A ETE MICROFILMEE TELLE QUE NOUS L'AVONS RECUE

Cette copie a été faite à partir d'une microfiche du document original. La qualité de la copie dépend grandement de la qualité de la thèse soumise pour le microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

NOTA BENE: La qualité d'impression de certaines pages peut laisser à désirer. Microfilmee telle que nous l'avons reçue.

Division des thèses canadiennes
Direction du catalogage
Bibliothèque nationale du Canada
Ottawa, Canada K1A 0N4

THE UNIVERSITY OF ALBERTA

METABOLIC FACTORS ASSOCIATED WITH TENSION DECLINE IN THE ELECTRICALLY STIMULATED IN SITU CANINE GASTROCNEMIUS MUSCLE

by

© PHILLIP F. GARDINER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSICAL EDUCATION

EDMONTON, ALBERTA

FALL, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Metabolic Factors Associated With Tension Decline in the Electrically Stimulated In Situ Canine Gastrocnemius Muscle", submitted by Phillip F. Gardiner in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

Howard A. Wenger
Supervisor

David C. Lucas

Leonard A. Kaufman

James Runco Jr.

A. H. Glassford

Ex Officio

D. L. Kilgore

External Examiner

Date October 21, 1976

DEDICATION

To Anita, Sandra and Michael, for having faith in a dreamer.

ABSTRACT

In order to investigate metabolic correlates to the characteristic pattern of tension decline seen with rhythmically working muscle, the surgically isolated, electrically stimulated dog gastrocnemius muscle group was used as an experimental model. Twenty-nine dogs served as experimental animals. The muscle group was surgically isolated and stimulated maximally using trains of impulses (5 ms D.C. square waves, 22 Hz, maintained for 0.7 s, 28 times per minute) delivered to the muscle belly. Estimates of arteriovenous differences of free fatty acids, glucose, lactate and plasma water were obtained at selected intervals along the tension-time curve. In addition, muscle concentrates of glycogen, ATP, CP, lactate, pyruvate, β-hydroxybutyrate and acetoacetate, were measured in freeze-clamped muscle samples taken when the muscle had attained a specified percentage of original tension.

The highest correlation between any single arteriovenous parameter and tension-time parameter was that between lactate A-V difference and time from beginning of stimulation ($r=.63$, $p<.001$). Lactate A-V difference was significantly correlated ($p<.01$) with all tension-time parameters, which was not the case with any other single A-V parameter. The highest correlation when relating muscle metabolite concentrations to tension-time parameters was between muscle lactate concentration and the rate of tension decline at sample time ($r=.80$, $p<.001$). Quantitative glycogen estimates revealed a gradual decline during the contraction period, while fibre glycogen utilization patterns illustrated a high rate of depletion in fast-twitch relative to slow-twitch fibres.

Estimates of mitochondrial $[NAD^+]/[NADH]$ ratios suggested well-oxygenated mitochondria throughout the contraction period, while

cytoplasmic [NAD^+]/[NADH] was significantly ($p < .05$) decreased at the period of most rapid tension decline (80%).

The relationships between lactate parameters and tension-time indices suggest that lactate presence in muscle tissue may exert an influence on the ability of muscle to maintain tension during continuous contractions. The dynamics of mitochondrial and cytoplasmic [NAD^+]/[NADH] ratios suggest that a major portion of the lactate produced was not due to muscle hypoxia, but to "aerobic glycolysis". It is postulated that the initial rapid decline in tension during maximal contractions is related to lactate influence on the contractile machinery, and that the final attainment of steady state tension is related to glycogen depletion.

ACKNOWLEDGEMENTS

It is with deepest gratitude that I thank Dr. Howard Wenger, not only for his endless enthusiasm and support, but also for his contagious philosophy of life. In addition, special appreciation is extended to Dr. David Secord, without whose boundless faith, energy and knowledge this project would still be in the conceptual stage.

I wish to thank the committee members, Dr. J.C. Russell, Dr. L.I. Wiebe and Dr. R.G. Glassford for their judicious contributions, and to Dr. D.W. ("Dee") Edington and Dr. A.W. ("Bert") Taylor for their extremely valuable, discerning input.

A special thanks is extended to Dr. K. Kowaleski, Director of the Surgical Medical Research Institute, for his support as well as his profound influence on my approach to scientific investigation, and to Mrs. M. McCubbin, Mr. T. Germaine and Mrs. J. Hantelmann.

Finally, I wish to thank my friend and colleague, Dr. Gary Ness, for his irreplacable assistance and contribution in the lab, and for his companionship during our many successful and unsuccessful attempts at stalking Alberta trout.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
METHODS AND PROCEDURES	5
Experimental Animals	5
Anesthesia	5
Surgical Isolation of Gastrocnemius Muscle Group	5
Preparation of Left Muscle Group for Stimulation	6
Measurement of Muscle Blood Flow	8
Measurement of Muscle Compartment Temperature	10
Measurement of Rectal Temperature	10
Measurement of Arterial Blood Pressure	11
Recording of Parameters	11
Prestimulation Period	11
Experimental Design	14
Muscle Group Stimulation	14
Blood Sampling Techniques	16
Tissue Sampling Techniques	16
Blood Analytical Techniques	18
Tissue Analytical Techniques	19
Calculations	20
Statistical Analysis	21
RESULTS	23
DISCUSSION	54
***	
REFERENCES	82
APPENDIX A. REVIEW OF LITERATURE	98

SECTION PAGE

APPENDIX B. NUTRITIVE CONTENT BURGER BITS 123

APPENDIX C. PLATES ILLUSTRATING PREPARATION 126

APPENDIX D. FORMULAE, NOMOGRAMS AND TISSUE LACTATE PILOT DATA 134

APPENDIX E. ANIMAL DESCRIPTION AND ASSIGNMENT, PRE-POST EXPERIMENTAL DATA, AND CORRELATION TABLES 140

APPENDIX F. MICROGRAPHS 158

LIST OF TABLES

Table	Description	Page
I	Muscle Tension Development and Times of Tissue Sampling	25
II	Blood Flow. Oxygen Consumption and Venous Blood Characteristics at Rest and at Different Relative Tension Levels During Contractions	27
III	Metabolite and Water Arteriovenous Dynamics at Rest and at Different Relative Tension Levels During Contractions	28
IV	Summary of Significant ($p < .01$) Correlations Between Arteriovenous and Tension-Time Parameters	31
V	Summary of Significant ($p < .01$) Correlations Among Arteriovenous Parameters	32
VI	Muscle ATP and CP Concentration in Right Control and Left Stimulated Muscles	36
VII	Muscle Glycogen Concentrations, Glycogen Ratios (L/R) and Total Glycogen Utilized in Right Control and Left Stimulated Gastrocnemius Muscles	37
VIII	Muscle Lactate and Pyruvate Concentration in Right Control and Left Stimulated Gastrocnemius Muscles	39
IX	Estimated Cytoplasmic and Mitochondrial $[NAD^+]/[NADH]$ Ratios in Right Control and Left Stimulated Gastrocnemius Muscles	40
X	Fibre Composition of Gastrocnemius Muscle (Medial Head)	42
XI	Summary of Significant ($p < .01$) Correlations Between Muscle Metabolite Concentrations and Tension-Time Parameters	45
XII	Comparisons of Selected Parameters Measured During the Level-Off Period in the 65%L and 50%L Groups	47
XIII	Arteriovenous Dynamics Measured Five Minutes Prior to the End of the Level-Off Period in 65%L and 50%L Groups	48
XIV	Muscle Metabolite Levels in the Left and Right Gastrocnemius Muscle in 65%L and 50%L Groups	50
XV	Relationships of Selected Parameters to Tension at Which Level-Off Occurred (% of Original) During Contractions	51

LIST OF FIGURES

Figures		Page
1.	Schematic Representation of Experimental Model	12
2.	Reproduction of Recorded Parameters at Various Intervals During a Typical Experiment	13
3.	Experimental Protocol	17
4.	Tension Development Changes of Combined Groups With Time in Maximally Stimulated Gastrocnemius Muscle Group	24
5.	Relationship Between Lactate (A-V) Difference and Elapsed Time From Beginning of Stimulation	33
6.	Relationship Between Arterial pO_2 and Oxygen (A-V) Difference	35
7.	Relationship Between Muscle Lactate Concentration and Rate of Tension Decline at Sample Time	46
8.	Relationship Between Lactate (A-V) Difference Measured at 65% and Tension at which Level-Off Occurred	52

INTRODUCTION

The investigation of the factors limiting human physical performance, and the adaptability of the human organism in attempting to offset these factors, has been the scope of a vast amount of research in this century. In particular, the study of the physical and biochemical occurrences in skeletal muscle in response to contractile activity constitutes a major area of endeavour aimed at determining the role of the muscle in imposing limitations to the total human organism during exercise. This area has, in itself, been the focus of several comprehensive texts and symposia (Simonson, 1971; Keul et al., 1972; Keul, 1973; Howald and Poortmans, 1975). The resulting proliferation of knowledge has served to emphasize the complexity of the response of muscle tissue to contraction, especially in light of the various forms, intensities and durations of contractions possible during exercise. This line of research has also posed the suggestion that the major limiting components to exercise of certain intensities and durations may reside in the muscle tissue itself, as opposed to limitations in the capabilities of the cardiovascular, respiratory, and other support systems. This latter possibility is especially lucrative in light of the research evidence generated within the last decade which illustrates the metabolic adaptability of muscle tissue to various kinds of chronic activity (Keul, 1972; Holloszy, 1975, Howald and Poortmans, 1975). It is an acknowledged fact that human voluntary exercise involves the interaction of working muscle tissue with the cardiovascular, respiratory and temperature regulating systems. However, the examination of the response of muscle to continued contraction seems fundamental to an understanding of its role in the chain of events leading to fatigue, especially in types of exercise where the primary limiting factor may reside in muscle tissue itself.

Improvements in techniques for the sampling and assay of muscle tissue within the last decade have resulted in the proposal of several hypotheses relative to muscle metabolic factors limiting continuous contractions. Factors such as depletion of high-energy phosphates (Hultman et al., 1967; Gubjarnason et al., 1970; Karlsson et al., 1971; Spande and Schottelius, 1971), depletion of fibre glycogen content (Gollnick et al., 1973; Armstrong et al., 1974; Gollnick et al., 1974), the accumulation of lactate and the concomitant decrease in muscle pH (Karlsson et al., 1971; Hermansen et al., 1972; Hirche et al, 1975; Hulten et al., 1975; Sahlin et al., 1975), alterations in muscle temperature (Brooks et al., 1971; Edwards et al., 1972; Saltin et al., 1972; Schafer, 1973) or electrolyte balance (Bergstrom et al., 1971; Hudlicka, 1973), and changes in the structural integrity of muscle enzymes (Hultman and Bergstrom, 1973; Sahlin et al., 1975) and mitochondria (Gollnick et al., 1971; Schafer, 1973; Gale, 1974; Gonzalez-Serratos et al., 1974) have all been put forth as possible muscle fatigue mechanisms during exercise.

Simonson (1971) has categorized these possible mechanisms into four fundamental processes: the accumulation of waste substances; the depletion of substances necessary for activity; changes in the physicochemical state of the substrate; and disturbance of regulation and coordination. It is now recognized that the primary factor in muscle which is limiting to continued performance may be specific to the intensity, duration, and the type of exercise considered. In addition, in view of the heterogeneity of fibre types that can exist in any

muscle (Peter et al., 1972), the limiting components may well be localized to a specific population of muscle fibres being recruited during that activity (Armstrong et al., 1975).

The investigation of the muscle metabolic factors which limit continuous exercise is plagued with technical and theoretical difficulties, especially when utilizing an exercising human or animal as the experimental model. Measurements under these conditions are, of necessity, taken at "volitional fatigue", and the point of cessation of exercise, as well as the level of blood and muscle metabolites, are dictated to some extent by the psychological sensations which have preceded the cessation. Although research in this area must eventually attempt to explain the physiological changes during exercise which produce the psychological desire to stop exercising, the "volitional" aspect of fatiguing exercise may be considered a confounding variable when examining the physiological changes alone. In addition, the use of voluntarily exercising animals or humans makes the extrication of muscle tissue samples of sufficient size or with sufficient speed too difficult to warrant adequate conclusions relative to the metabolic state of the muscle at fatigue. This is especially significant in the case of the more viable metabolites, such as ATP and CP (Lowry and Passonneau, 1972).

The investigation of metabolic occurrences in isolated mammalian muscle preparations, electrically stimulated to elicit rhythmic contractions, has furthered our understanding of muscle energy flux during increased contractile activity (Stainsby and Otis, 1964; Kugelberg and Edstrom, 1968; DiPrampero et al., 1969; Edgerton et al., 1970; Burke et al., 1971; Baldwin and Tipton, 1972; Edington et al., 1973; Hirche

et al., 1975). However, no systematic approach has been used to attempt to relate crucial arteriovenous and muscle metabolic changes with simultaneous measurements of the tension decline of a rhythmically contracting muscle.

Purpose of the Study

The purpose of the investigation was to determine the metabolic basis for the reversible tension decline of an in situ muscle preparation during elicited rhythmic contractions. More specifically, estimates of the blood and muscle concentrations of specific metabolites, as well as muscle fibre recruitment patterns, were monitored during the working period, and related to tension decrement patterns.

METHODS AND PROCEDURES

Experimental Animals

Twenty-nine healthy mongrel dogs were acquired as unclaimed dogs through the municipal dog pounds, under the authority of provincial legislation. Before being used as an experimental animal, each dog had been quarantined for 2 weeks at the University of Alberta Animal Center. During this period each dog was vaccinated against canine distemper and hepatitis, bathed, deflead and wormed. Dogs were maintained on a diet of Burger Bits (Standard Brands, Montreal; Appendix B) and water was administered ad libitum. Each animal was fasted for 24 hours before the experimental procedure.

Anesthesia

All experimental animals were anesthetized with halothane (Fluothane, Ayerst, McKenna and Harrison Ltd., Montreal). Initial induction was achieved via a 5% mixture of halothane and air (5 litres per min) administered through a nose-cone to the restrained animal. Following induction, the dog was intubated, and anesthesia was maintained with a 1.5 to 2.0 per cent mixture of halothane and air (1.5 to 2.0 litres/min).

Surgical Isolation of Gastrocnemius Muscle Group

Following shaving of all surgical sites, an incision was made on the medial aspect of both hindlimbs from the stifle joint to the achilles tendon, approximately three centimeters proximal to the calcaneus, and the skin retracted. The branches of the medial saphenous artery and vein imbedded in the superficial fascia were doubly ligated with cotton and severed between the ties. The remaining fascia and connective tissue were dissected away, exposing the superficial

musculature.

The gracilis, medial sartorius, semitendinosus, and the two bellies of the semimembranosis muscles were bluntly dissected away from surrounding fat and connective tissue, doubly ligated near the insertions on the tibia, and severed between cotton ties.

The venous drainage from the gastrocnemius-flexor digitorum superficialis muscle group was isolated by sequentially ligating all venous connections with the popliteal vein which were not draining the muscle group specifically. The arterial supply to this area was left intact. X-rays of similarly isolated gastrocnemius muscles infused with 30% Hypaque (Winthrop Laboratories, Montreal) attested to the success of this procedure in effectively isolated muscle venous drainage (Appendix C, Plate I).

The branch of the sciatic nerve innervating the gastrocnemius-flexor group was freed of fat, doubly ligated approximately 3 cm from the caudal border of the gastrocnemius muscle with cotton, and severed between the ties. The muscle groups of both legs were freed as much as possible from surrounding connective tissue (Appendix C, Plate IIA).

Throughout the surgical procedures bleeding was controlled using an electrical fulguration unit (Birtcher Electro-surgical Unit, Model 755).

Following the initial surgical procedures on both hind legs, the wounds were packed with saline-soaked gauze, and closed with towel clamps.

Preparation of Left Muscle Group for Stimulation

An incision approximately 8 cm in length was made along the achilles tendon, from a point 4 cm proximal to the calcaneus, distally

over the calcaneus and along the continuation of the tendon of the flexor digitorum superficialis toward the hindpaw. The calcaneus and achilles tendon were freed from underlying fat and connective tissue by blunt dissection. The calcaneus was hand drilled with a 1/8 inch bit, perpendicular to the plane of the achilles tendon, and the drill bit was left in place. The calcaneus was then severed from the hindpaw with a bonesaw, thereby disconnecting the gastrocnemius-achilles-calcaneus system from the hindpaw.

The femur directly above the origin of the gastrocnemius-flexor muscle group was drilled transversely with a 1/4 inch drill bit (10.5 inches in length) and the drill bit left in place.

A specially designed clamp (Appendix C, Plate IIIA) to which was attached a 1/8 inch steel cable, was used to clasp the ends of the drill in the calcaneus. A hook at the end of the cable was attached to a myograph-linear displacement transducer complex (Appendix C, Plate IIIA). The femur drill was clamped on both sides of the femur using clamps screwed onto the ends of 1/2 inch steel rods. These rods were in turn clamped to 2 upright 1/2 inch steel rods bolted firmly to a platform of 3/4 inch spruce plywood. A turnbuckle strut was placed between one of the upright rods and the myograph post in an attempt to minimize flexing of the units during high-tension contractions (Appendix C, Plate IIIB).

During the process of fixing the femur, an attempt was made to mimic as closely as possible the natural angle of the knee and hip joint, in order to ensure adequate femoral arterial and venous blood dynamics. Once the femur was fixed, the steel cable attached to the calcaneus was hooked to the myograph-linear displacement transducer

system and the muscle loaded with a tension of 1 kilogram. All clamps were then tightened with pliers (Appendix C, Plate IVA).

The myograph-linear displacement transducer system was calibrated periodically by hanging a preweighed bucket, containing water of known volume and temperature, from the myograph bar using a pulley and cable. The response of this system was linear ($\pm$ 2%) up to a force of 20 kg.

During extensive pilot experiments, attempts were made to determine to what degree shifting of components in the tension monitoring system was contributing to the apparent decline in tension development of the muscle group during continuous contractions. Recovery experiments indicated that, following a 30 minute recovery period, resumption of stimulation elicited muscle tensions which reproduced in magnitude those which had occurred during the first minute of the initial contraction period, even when the previous contraction period had decreased the initial tension development to 50 per cent. In addition, in no experiment was there evidence of a decrease in the baseline tension, which one would expect to occur if shifting of components in the system were to take place. On the basis of these results it was concluded that any change in the tension recording was due solely to alterations in the ability of the muscle group itself to maintain the initial tension development during continuous contractions.

Measurement of Muscle Blood Flow

Measurement of the flow of venous blood draining the gastrocnemius-flexor muscle group was performed via an electromagnetic square-wave flowmeter (Model SWF-1M, Zepeda Instruments, Seattle, Washington) with a 3 mm flow-through probe. The left cephalic vein was isolated and catheterized with polyethylene tubing (I.D. 2.5 mm) filled with saline

and stoppered with a three-way stopcock, which was subsequently used as a venous sampling port. The stopcock was then attached to the outflow side of the flow probe with polyethylene tubing (I.D. 4.0 mm) and the stopcock was closed.

The exposed femoral vein was occluded and catheterized with polyethylene tubing (I.D. 2.5 mm) such that the mouth of the catheter was situated approximately at the junction of the popliteal and femoral veins. The femoral vein was tied proximal to the entry of the catheter, and the catheter secured with a cotton ligature (Appendix C, Plate IB). The opposite end of the catheter was immediately attached to the inflow side of the flow probe and the occlusion to the vein released, allowing the venous blood from the muscle to flow through the catheter, through the flow probe, and return to the animal via the cephalic vein. The time of occlusion of venous flow was 30 to 40 seconds. The drop in vertical distance from the catheterized femoral vein to the flow probe was approximately 12 to 15 cm, and the probe was horizontally level with the entrance of the outflow catheter into the cephalic vein (Appendix C, Plate IIB).

Blood was rendered incoagulable with an initial dose of heparin (Sigma Chemical Co., St. Louis, Mo.) of 500 units/kg, with additional doses of 200 units/kg every hour.

The flow meter was calibrated during each experiment by timed collections of blood into a graduated cylinder through the venous sampling port. The meter was zeroed by blood flow occlusion using the stopcock. Preliminary calibration of the flow meter before the experimental series illustrated the meter to yield a linear response up to flow rates approximately 200 ml/min. Although there appeared to be some

electrical interference with the flow signal when relatively high stimulation voltages were used for muscle stimulation, the interference was minor, and appeared to fluctuate around the true signal.

Measurement of Muscle Compartment Temperature

Muscle compartment temperature was monitored using a copper-constantan thermocouple imbedded in a 19 gauge needle grounded to the recorder. The coupler contained an internal reference junction. The thermocouple was calibrated with water of known temperatures.

In the initial pilot experiments, the needle thermocouple was imbedded in the muscle belly to obtain a record of internal muscle temperature. However, in view of the bleeding that occurred from the resulting muscle wound during the longer contraction periods, it was felt that damage to the muscle tissue might affect muscle performance. For this reason, during the experimental series, the thermocouple probe was placed in the cavity which resulted from the blunt dissection of the muscle group from the small muscles lying along the tibia. As a result, a measure of the temperature of the compartment itself, as opposed to actual internal muscle temperature, was obtained.

An infrared lamp focussed on the covered limb was used to bring the muscle compartment temperature to rectal temperature during the prestimulation period.

Measurement of Rectal Temperature

Rectal temperature was monitored via a mercury thermometer inserted in the dog's rectum to a depth of 14 cm. Core temperature was maintained at 37 to 39°C using an adjustable heating pad secured to the dog's chest and upper abdomen.

Measurement of Arterial Blood Pressure

Arterial blood pressure was monitored continually by means of a Statham pressure gauge (Statham, Hato Ray, Puerto Rico). The pressure measured was that of the carotid artery, which was isolated and catheterized with polyethylene tubing (I.D. 2.5 mm) filled with saline and attached to the transducer.

Throughout the experiment, an attempt was made to maintain initial blood pressure levels using an intravenous drip of Ringers solution into the right cephalic vein, at a rate of 4 to 6 ml/min.

The pressure transducer was calibrated regularly using a manometer (Thistle, Model C80216).

Recording of Parameters

A schematic representation of the experimental model is presented in Figure 1.

The parameters of muscle tension development, muscle venous blood flow, muscle compartment temperature, and carotid arterial blood pressure were recorded continually on a 4-channel Beckman Type R Dynograph (Beckman Instruments Inc., Offner Division, Schiller Park, Ill.) (Figure 2).

Prestimulation Period

After the preparatory procedures, the muscle group was allowed to rest for at least 30 minutes, and until muscle blood flow had stabilized and muscle compartment temperature was equal to rectal temperature. During this period the muscle group was covered with saline soaked gauze and a slow continuous drip of isotonic saline was applied to the gauze to keep the compartment moist. Excess saline was drained from the muscle compartment by means of a 12 gauge needle inserted

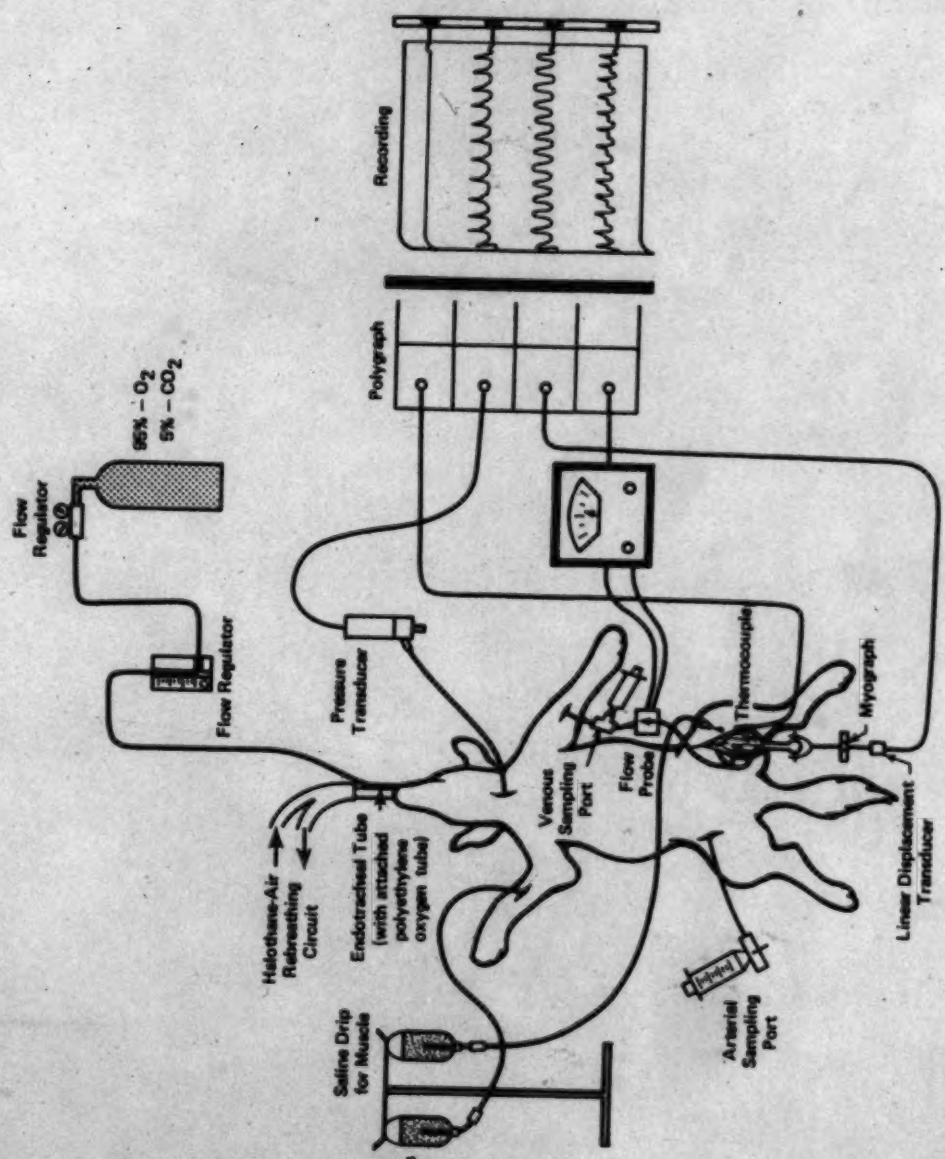


Figure 1: Schematic Representation of Experimental Model

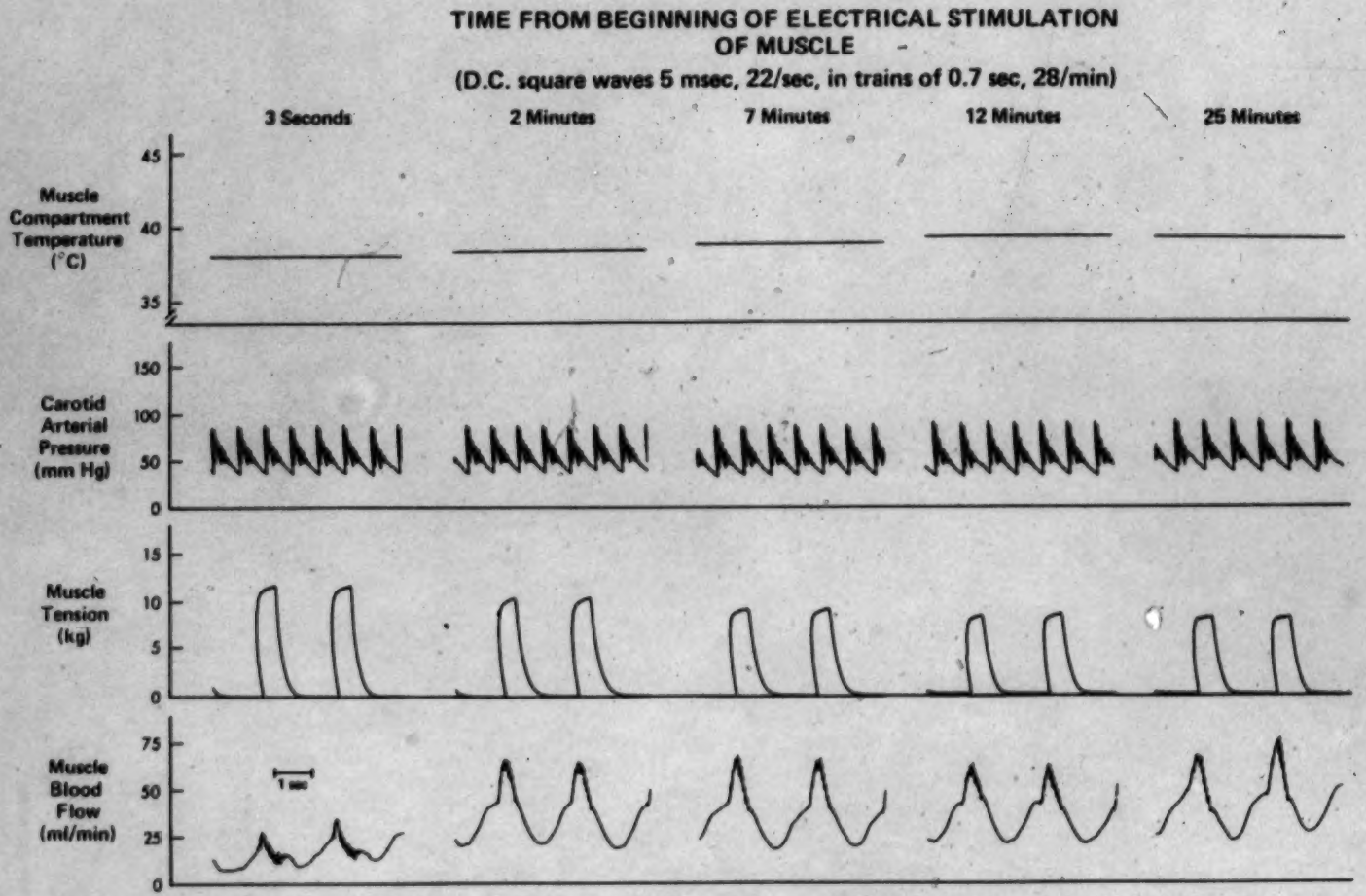


Figure 2: Reproduction of Recorded Parameters at Various Intervals During a Typical Experiment

through the skin on the lateral aspect of the compartment (Appendix C, Plate IIIB).

In addition, a supplement of 95% O_2/5% CO_2 to the anesthetic mixture was initiated during this period. The oxygen was administered via a polyethylene tube (I.D. 2.5 mm) which was attached to the endotracheal tube along its length, at a rate of 500 to 1000 ml/min. Pilot studies indicated that this oxygen supplement, administered at a rate which was proportional to the weight of the dog, could be controlled to elicit arterial pO_2 values of 80 to 110 mmHg.

Experimental Design

During the calibration and prestimulation period, each dog was randomly assigned to one of four conditions, depending on the level of tension decline to which the muscle group was to be subjected. In the "80%" group, the muscle group was stimulated continuously until the tension development during contractions had decreased to 80% of the tension occurring upon initiation of contractions. Two other groups were similarly designated as "65%" and "50%" groups. A fourth group was designated as a "level-off" condition (50%L), in which the muscle was stimulated to contract continuously until the change in tension was less than 5% over a 30 minute period. This levelling off was witnessed to occur at approximately 50% in pilot experiments; therefore, the difference between the 50% group and the 50%L group was the time during which the muscle group worked at this level. It was hoped that the incorporation of both a "50%" and "level-off" group would aid in distinguishing between tension- and time-associated metabolic changes.

Muscle Group Stimulation

The muscle group was stimulated to contract using an Electronic

Stimulator (Model 751, American Electronic Labs, Inc., Philadelphia, Penn.), modified to yield trains of impulses. The stimulating electrode consisted of a pair of 1 mm stainless steel wires which were inserted through the mid-portion of the muscle belly approximately 2 cm apart. Pilot experiments which were conducted using electrical stimulation to the distal nerve stump versus direct stimulation to the muscle belly presented doubt as to the maintenance of viability of the nerve during the longer stimulation periods; therefore, direct muscle stimulation was employed throughout the experimental series.

The delivery of impulses to the muscle was in the form of trains, lasting for 0.7 seconds, each of which was composed of 5 msec duration impulses delivered at a rate of 22 per second. One contraction occurred every 2.2 seconds; therefore, the muscle contracted 28 times per minute tetanically. Muscle shortening during high tension contractions was no larger than 5 mm.

This pattern of muscle stimulation was chosen for a number of reasons. It has been speculated that a high proportion of _in vivo_ muscle contractions are most likely tetanic, as opposed to twitch, in nature (Stainsby and Barclay, 1972; Barclay _et al._, 1974). In addition, pilot studies indicated that this contraction pattern resulted in muscle metabolic levels which were primarily aerobic, as evidenced by the relatively low venous lactate values and oxygen uptake levels below reported maximum values (Welch and Stainsby, 1967; Hirche _et al._, 1975). Finally, this contraction pattern resulted in tension decrease curves which allowed sufficient time for blood and tissue sampling during the initial period of rapid tension decline, while at the same time keeping the total time of the longer experiments to approximately 90 to 120 minutes.

Blood Sampling Techniques

Figure 3 illustrates the protocol for the collection of muscle and blood samples during each of the 4 experimental conditions.

During the prestimulation period and at the designated intervals during the contraction period, 5 ml samples of femoral venous blood were collected into 5 or 10 ml glass syringes lubricated with paraffin oil via the stopcock in the venous circuit. Each sample was collected at a rate which approximated that indicated by the preceding uninterrupted blood flow recording. The syringe was capped and immediately transported to the adjacent lab for processing. Arterial blood samples were taken through a small polyethylene catheter (I.D. 1.0 mm) inserted in the right cranial femoral artery. The sampling end of the catheter was fitted with a 3-way stopcock. Arterial samples (5 ml) were drawn at the same times as venous samples.

Tissue Sampling Techniques

At the end of each experiment, tissue samples were taken from the experimental left muscle and from the contralateral right muscle for the quantitative measurement of muscle metabolites and the qualitative assessment of muscle fibre composition and recruitment. Since the tissue concentrations of the muscle metabolites measured (ATP, CP, lactate, pyruvate, acetoacetate, β-hydroxybutyrate) can change drastically upon cessation of exercise (Ahlborg et al., 1972; Lowry and Passonneau, 1972), it was deemed necessary to stop all metabolic processes as soon after contraction as possible. For this purpose, a pair of tongs was designed which, when cooled to liquid N_2 temperature, would cool a 1.0 to 1.5 gram clamped piece of tissue to -50°C within 1.5 seconds. At the time of sampling, the precooled tongs were used

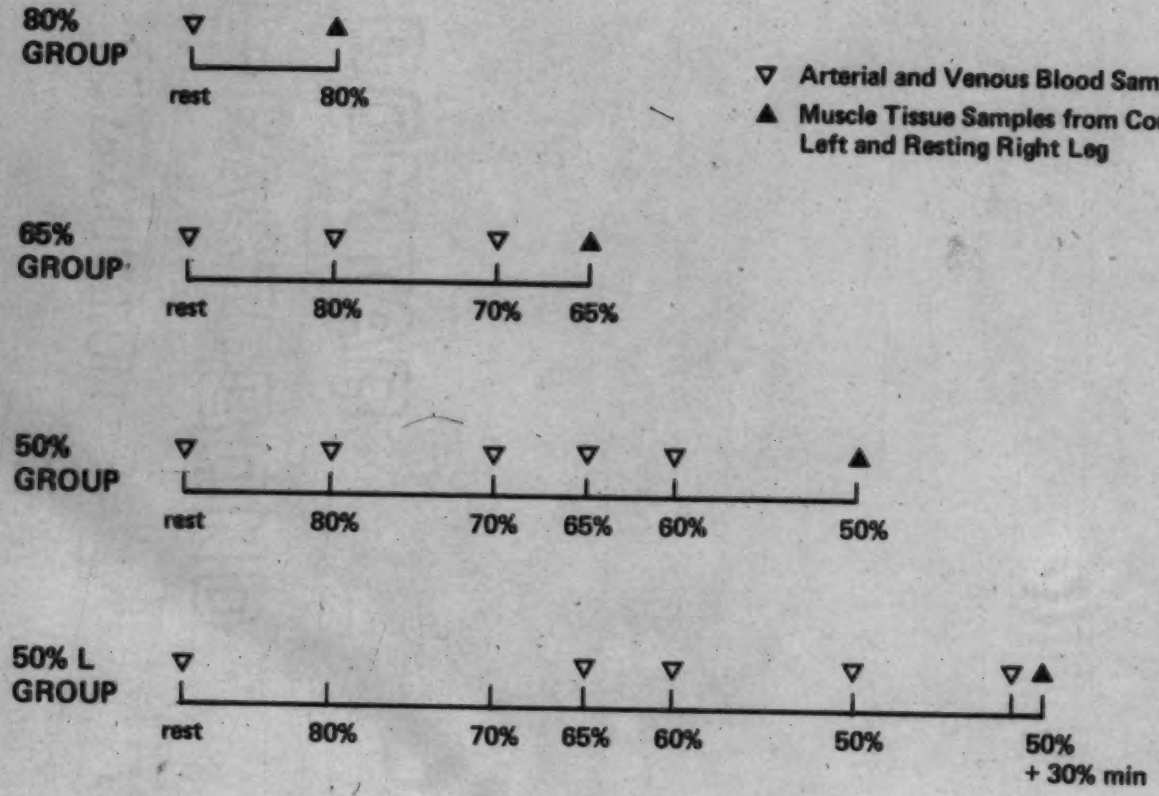


Figure 3: Experimental Protocol

to clamp a portion of the medial head of the left gastrocnemius muscle. Once clamped, the tissue within the tongs was dissected free from the rest of the muscle with a scalpel. The tongs, with the sample, were plunged immediately into liquid N_2 for 10 to 15 seconds, after which time the sample was transferred to a polyethylene vial which had been precooled in dry ice. The vial was capped and set in a covered Dewar flask containing dry ice until being transferred to a low temperature cabinet maintained at -60°C. Pilot studies using a thermocouple imbedded in the muscle to be sampled illustrated that the time period between the cessation of a contraction and cooling of the clamped tissue sample to -50°C was approximately 1.5 to 2.0 seconds. A freeze-clamped tissue sample was taken from the contralateral right muscle immediately following left muscle sampling.

After collecting the freeze-clamped samples, the remaining portion of the entire muscle group from each leg was excised and placed in saline. The medial and lateral heads of the gastrocnemius, and the flexor digitorum superficialis muscle, were separated, trimmed of fat, blood vessels and tendon, and weighed. A portion from the mid-belly from each head of the muscle group was removed, cooled for 15 seconds in isopentane cooled to its freezing point in liquid N_2, placed in test tubes precooled in dry ice, and stoppered. The test tubes were stored in a covered Dewar flask containing dry ice until transfer to a low temperature cabinet maintained at -60°C.

Blood Analytical Techniques

Paired arterial and venous blood samples were analyzed for pO_2, pCO_2, and pH (Radiometer blood gas apparatus - pH meter 27, Radiometer Co., Copenhagen), hemoglobin (cyanomethemoglobin technique, Davidsohn

and Henry, 1969) and hematocrit (microhematocrit technique) immediately following transport of samples to the lab. A portion of whole blood (1 ml) was deproteinized in 8% cold perchloric acid for analysis of blood lactate (Sigma Chemical Company, 1968). The remaining portion of each blood sample was centrifuged, and the plasma frozen. Subsequent analyses for plasma glucose (Sigma Chemical Company, 1973) and plasma free fatty acids (Pinelli, 1973) were performed on the thawed plasma.

Tissue Analytical Techniques

The freeze-clamped tissue plugs were trimmed of tissue which had not been clamped, were weighed, and transported in liquid nitrogen to a cold room maintained at 4°C. The plugs were ground to powder using a porcelain mortar and pestle at liquid nitrogen temperature, and perchloric acid extracts of the samples were made according to Lowry and Passonneau (1972). The extract from each tissue sample was stored in 6 to 8, 3 ml polyethylene snap-top tubes at -60°C until analysis.

Analysis of tissue ATP, CP, lactate and pyruvate were performed on separate samples of extract according to Lowry and Passonneau (1972). The extracts were also analyzed for acetoacetate and β-hydroxybutyrate concentration using the methods modified from Edington, Ward, Saville (1973). The latter techniques were modified for larger volumes, and β-hydroxybutyrate was analyzed using Tris buffer at pH 8.5. Measurements of reactions were performed at 340 nm using a Pye Unicam SP 1800 spectrophotometer (Pye Unicam Ltd., Cambridge, England).

Muscle glycogen analysis was performed on 35 to 50 mg samples of muscle taken from the isopentane-cooled samples, employing the method of Lo, Russell and Taylor (1970).

The isopentane-cooled frozen tissue samples were transported to

an Ames Lab-Tek microtome-cryostat (Ames Lab-Tek, Inc., Westmont, Ill.) where they were allowed to warm to cryostat temperature (-20°C). Serial sections 10 and 16 microns in thickness were cut and mounted on 25 x 75 mm microscope slides and allowed to dry overnight. Sections were incubated for the demonstration of myosin ATPase (10 micron section) and NADH diaphorase (10 micron section) activities according to Dubowitz and Brooke (1973). The periodic acid-Schiff (PAS) technique to illustrate glycogen content was performed according to Dubowitz and Brooke (1973) on the 16 micron sections. Fibres were classified according to speed of contraction, from the myosin ATPase stain, and oxidative capacity, from the NADH diaphorase stain, using the criteria suggested by Peter et al. (1972).

Calculations

To determine muscle oxygen uptake, arterial and venous pO_2 values were first converted to % hemoglobin saturation using the nomogram of Astrup et al. (1965). Oxygen content of arterial and venous blood was then determined from the arterial hemoglobin values, and oxygen uptake determined using the Fick principle (Rushmer, 1970). The Astrup et al. nomogram and formulae used for oxygen uptake determination are presented in Appendix D.

To determine respiratory quotient (R.Q.) total CO_2 content of arterial and venous blood was determined from pCO_2 and blood pH values using the Siggaard-Andersen Alignment Nomogram (Siggaard-Andersen, 1963). Respiratory quotient was then calculated using the formula as outlined by Consolazio, Johnson and Pecora (1963). The Siggaard-Andersen Alignment Nomogram and the formula for R.Q. determination are included in Appendix D.

The determination of arteriovenous differences of all blood metabolites was performed using the correction for plasma water loss suggested by Schlein et al. (1973). The formula used is included in Appendix D.

Cytoplasmic and mitochondrial $[NAD^+]/[NADH]$ ratios were determined using the formulae suggested by Williamson et al. (1969) and presented in Appendix D.

All muscle concentration and arteriovenous differences were expressed per unit of wet tissue weight.

Statistical Analysis

The significance of differences between pre- and post-stimulation control parameter means was estimated using t-test for correlated samples. T-tests for independent samples were used to test for differences between arteriovenous measurements, and between control and stimulated muscle metabolite concentrations. Single-factor analysis of variance was used to test for significant differences among control muscle and stimulated muscle means across groups.

Separate correlation matrices were constructed for arteriovenous and tissue metabolite parameters in an attempt to determine the degree of relationship between these variables and muscle tension parameters.

All calculations were performed by an IBM 360/67 computer. Programmes for statistical analyses were taken from DERS computer programme library (Department of Educational Research Services, University of Alberta).

Post Hoc Data Considerations

During some of the experiments randomly assigned to the 50%L condition, it was found that level-off tension occurred at relative tensions significantly higher than 50%. These experiments were subsequently

combined as a 65%L group, since the mean level-off tension approximated 65%. Comparisons of arteriovenous and muscle metabolite parameters were made between the 65%L and 50%L groups in an attempt to determine possible metabolic correlates to this difference in level-off tension.

RESULTS

A general description (age, weight, sex, breed, and assigned experimental condition) of each dog employed during the experimental series is contained in Appendix E, Table XVI.

The tension development-time relationship of the stimulated gastrocnemius-flexor muscle group is illustrated in Figure 4. The muscle group elicited an initial high rate of tension decrement, followed by a slower tension decline until an apparent "steady state" of muscle tension development occurred.

The data in Table I are a summary of the mean tension levels and times at which muscle samples were taken for each experimental group. During the experimental series, it was noted that, in some preparations randomly assigned to the 50%L condition, the elicited tension during continuous contractions levelled off at a tension development level which was higher than 50%. These experiments were subsequently grouped together as a "65%L" group as opposed to the "50%L" group, since the mean level-off tension closely approximated 65% (Table I).

T-tests for correlated samples were conducted on selected control parameters measured at the beginning and end of the level-off experiments. A summary of these data is contained in Appendix E, Table XVII. Significant changes with time were noted in arterial pCO_2, arterial blood pH, diastolic and systolic blood pressures, arterial lactate and free fatty acids, and arterial hematocrit. However, subsequent analyses performed relating these changes to tension parameters suggested lack of any significant relationships (Tables XX and XXI).

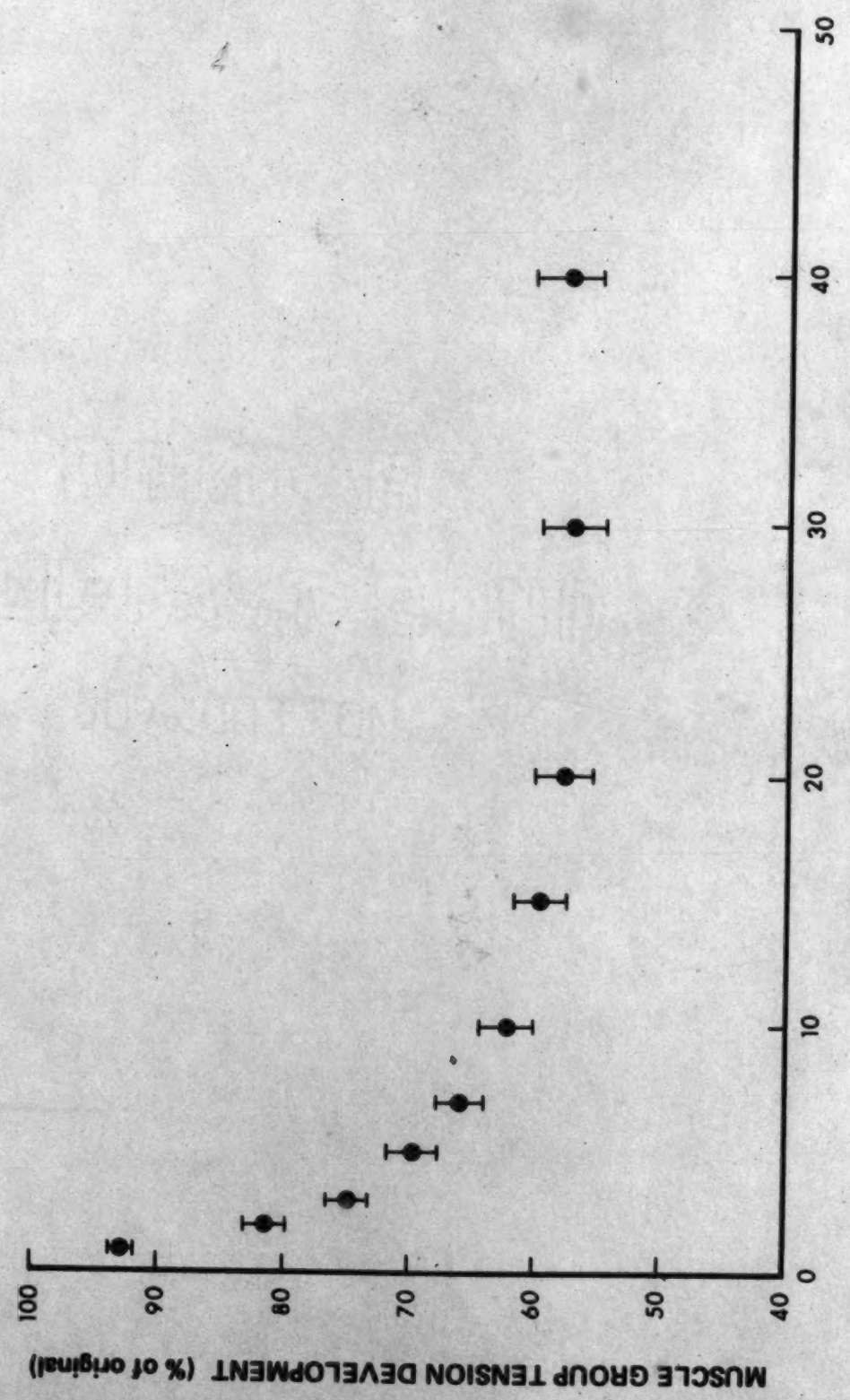


Figure 4: Tension Development Changes of Combined Groups with Time in Maximally Stimulated Gastrocnemius Muscle Group ($\bar{X} \pm SEM$)

GROUP	MUSCLE TENSION DEVELOPMENT AT END OF STIMULATION (% OF ORIGINAL)	TIME FROM BEGINNING TO END OF STIMULATION (MINUTES)
80% (N=5)	79.0 ± 0.8	3.2 ± 0.4
65% (N=5)	62.9 ± 1.2	9.1 ± 2.6
50% (N=6)	48.9 ± 1.5	45.7 ± 13.4
50%L (N=7)	49.9 ± 1.7	86.7 ± 7.8
65%L (N=6)	66.3 ± 2.0	82.4 ± 4.6

TABLE I MUSCLE TENSION DEVELOPMENT AND TIMES OF TISSUE SAMPLING[a]

[a] Values are $\bar{X} \pm$ SEM

ARTERIOVENOUS DYNAMICS DURING CONTINUOUS CONTRACTIONS

Arteriovenous dynamics at rest and at different tension levels during contraction are summarized in Tables II and III.

As a result of initiation of continuous contractions, muscle blood flow increased approximately 3.5 times the level recorded during the prestimulation period (Table II). The highest mean blood flow occurred at the 70% measurement interval, although statistically there were no differences among the contraction means ($p < .05$). All blood flow means during the stimulation period were significantly higher ($p < .01$) than the corresponding resting means.

Muscle oxygen uptake increased from rest to a maximum value at 80% of original tension development, which was approximately 12 times the resting mean (Table II). From 80%, there tended to be a gradual decrease in oxygen uptake throughout the remainder of the contraction period, although statistically the means were not significantly different ($p > .05$). All oxygen uptake means during the contraction period were significantly higher ($p < .01$) than the corresponding resting means.

Oxygen arteriovenous differences measured during the contraction period were all significantly higher ($p < .05$) than the corresponding resting means (Table II). The trend of oxygen (A-V) data tended to mimic that for muscle oxygen uptake in that the highest value was recorded at the 80% level, after which a steady decline in (A-V) oxygen difference appeared to occur.

In the venous blood draining the muscle group, initiation of contractions resulted in a decrease in pO_2 and pH, which remained significantly lower ($p < .01$) than the corresponding resting means, at all tension development levels (Table II). The decrease in both of these

PARAMETER	TENSION LEVEL (% OF ORIGINAL)						
	REST	80%	70%	65%	60%	50%	50%L
Muscle blood flow (ml/g/min)	0.22 ± 0.02	0.66 ± 0.08**	0.73 ± 0.12**	0.63 ± 0.06**	0.64 ± 0.06**	0.65 ± 0.07**	0.64 ± 0.07**
Muscle oxygen uptake (ul/g/min)	7.1 ± 0.7	84.0 ± 9.0**	83.7 ± 8.5**	79.2 ± 9.4**	78.6 ± 8.0**	63.8 ± 6.8**	58.3 ± 6.2**
Oxygen (A-V) difference (mm Hg)	37.8 ± 1.0	66.4 ± 2.8**	63.3 ± 4.1**	62.6 ± 2.4**	59.9 ± 3.4*	52.5 ± 3.4**	52.2 ± 3.7**
Venous blood pO_2 (mm Hg)	47.5 ± 1.4	24.2 ± 1.7**	25.1 ± 1.8**	27.7 ± 2.2**	27.6 ± 2.8**	34.8 ± 4.0**	32.0 ± 4.0**
Venous blood pCO_2 (mm Hg)	37.8 ± 1.0	66.4 ± 2.8**	63.3 ± 4.1**	62.6 ± 2.4**	59.9 ± 3.4**	52.5 ± 3.4**	52.2 ± 3.7**
Venous blood pH	7.31 ± 0.01	7.16 ± 0.01**	7.18 ± 0.02**	7.18 ± 0.01**	7.18 ± 0.01**	7.21 ± 0.01**	7.20 ± 0.02**
Respiratory Quotient (R.Q.)	1.14 ± 0.13	1.16 ± 0.15	0.84 ± 0.11	1.14 ± 0.08	0.99 ± 0.11	0.91 ± 0.16	0.80 ± 0.12

TABLE II BLOOD FLOW, OXYGEN CONSUMPTION AND VENOUS BLOOD CHARACTERISTICS AT REST AND AT DIFFERENT RELATIVE TENSION LEVELS DURING CONTRACTIONS[a]

[a] Values are $\bar{X} \pm$ SEM

* Significantly different from corresponding resting mean ($p < .05$)

** Significantly different from corresponding resting mean ($p < .01$)

PARAMETER	TENSION LEVEL (% OF ORIGINAL)						
	REST	80%	70%	65%	60%	50%	50%L
Lactate (A-V) difference (uM/g/min)	-0.05 ± 0.02	-0.72 ± 0.07**	-0.53 ± 0.10**	-0.50 ± 0.06**	-0.50 ± 0.09**	-0.38 ± 0.23	-0.07 ± 0.05
Glucose (A-V) difference (uM/g/min)	0.03 ± 0.02	-0.03 ± 0.09	0.11 ± 0.11	0.10 ± 0.06	0.35 ± 0.15	0.21 ± 0.06*	0.15 ± 0.20
Free fatty acids (A-V) difference (neq/g/min)	-20.1 ± 10.1	50.1 ± 50.1	76.2 ± 60.8	6.4 ± 26.3	-59.3 ± 83.1	9.9 ± 14.0	8.8 ± 71.3
Water (A-V) difference (ul/g/min)	1.1 ± 1.1	19.9 ± 5.4**	15.3 ± 4.8**	12.5 ± 4.8**	-2.8 ± 5.2	4.3 ± 3.1	5.0 ± 8.4

TABLE III METABOLITE AND WATER ARTERIOVENOUS DYNAMICS AT REST AND AT DIFFERENT RELATIVE TENSION LEVELS DURING CONTRACTIONS[a]

[a] Values are $\bar{X} \pm$ SEM

* Significantly different from corresponding resting mean ($p < .05$)

** Significantly different from corresponding resting mean ($p < .01$)

parameters was most pronounced at the 80% sampling interval, with subsequent venous pO_2 and pH means gradually increasing towards the corresponding resting means as contraction continued.

Venous pCO_2 was significantly higher ($p < .01$) than rest at all sampling intervals during contraction (Table II). As with the pO_2, pH, oxygen uptake and oxygen (A-V) difference, the highest deviation from the resting state was witnessed at 80% of original tension development.

Analysis of the respiratory quotient (R.Q.) data demonstrated no differences between any of the contraction means and the corresponding resting means, although there was a tendency towards a decrease in R.Q., compared to rest, in the longer experiments (Table II).

The muscle demonstrated a slight lactate output during the pre-stimulation period (Table III). Upon commencement of contractions, lactate output increased significantly ($p < .01$) to a level which was approximately 14.5 times that measured during rest. Lactate output at subsequent sampling times illustrated a gradual decline towards resting levels. By the 50% sampling interval, lactate output had decreased to a level which was not significantly different from the resting mean ($p > .05$).

The muscle demonstrated a slight blood glucose uptake during the pre-stimulation period (Table III). A glucose uptake significantly higher than rest ($p < .01$) was witnessed at the 50% sampling interval. The glucose uptake levels measured at all other sampling intervals, except for 80%, illustrated a tendency towards levels which were higher than the corresponding resting means. At 80%, a glucose output, not significantly different ($p > .05$) from the resting mean, was recorded.

Free fatty acid dynamics across the muscle failed to illustrate

any discernible trend when considered as a function of muscle tension development and showed a high degree of variation (Table III).

The muscle took up slight amounts of plasma water during the pre-stimulation period (Table III). Upon initiation of contractions, the muscle began to accumulate relatively large amounts of plasma water, as witnessed by the significantly higher ($p < .01$) positive water (A-V) difference measured at 80%. This accumulation decreased to smaller, but significant ($p < .01$) A-V differences at the 70% and 65% levels. By the 60% sampling interval, water (A-V) difference was not significantly different ($p > .05$) from the resting mean, and remained statistically unchanged from rest throughout the remainder of the contraction period.

Correlations Between Arteriovenous and Tension-Time Parameters

A summary of significant correlations ($p < .01$) is contained in Table IV. The entire correlation matrix is included in Appendix E.

The highest correlation between any single arteriovenous parameter and tension parameter was between arteriovenous lactate and time ($r=.63$, $p < .01$)(Figure 5). Lactate arteriovenous difference was significantly correlated ($p < .01$) with all other tension parameters, although correlation coefficients were lower than the coefficient of lactate (A-V) difference with time. It was noted that the parameters of systolic blood pressure, diastolic blood pressure, and arterial pO_2 were not significantly correlated with any tension parameters ($p > .05$).

Correlations Among Arteriovenous Parameters

Significant correlations ($p < .01$) among individual arteriovenous parameters are presented in Table V. The highest single correlation was between arterial hemoglobin concentration and arterial hematocrit ($r=.97$, $p < .01$). Arteriovenous O_2 difference (mmHg) and arterial pO_2

PARAMETERS CORRELATED	NUMBER OF OBSERVATIONS	r	PROBABILITY THAT r=0
LACTATE (A-V) DIFFERENCE (uM/g/min) VS:			
Time from beginning of stimulation (min)	75	.63	.001
Muscle tension development (kg)	75	-.41	.001
Muscle tension development (% of original)	75	-.37	.001
Muscle tension development (g/g)	75	-.37	.001
Rate of tension decline (g/g/min)	75	-.37	.001
Rate of tension decline (%/min)	75	-.35	.002
Rate of tension decline (g/min)	75	-.35	.002
MUSCLE BLOOD FLOW (ml/g/min) VS:			
Rate of tension decline (g/min)	74	-.35	.002
Rate of tension decline (%/min)	74	-.34	.003
Muscle tension development (kg)	74	-.34	.004
Rate of tension decline (g/g/min)	74	-.33	.004
VENOUS BLOOD pH VS:			
Rate of tension decline (%/min)	75	-.42	.001
Rate of tension decline (g/min)	75	-.41	.001
Rate of tension decline (g/g/min)	75	-.39	.001
Muscle tension development (kg)	75	-.30	.009
VENOUS BLOOD pCO_2 (mm Hg) VS:			
Muscle tension development (kg)	75	.61	.001
Muscle tension development (% of original)	75	.46	.001
Time from beginning of stimulation (min)	75	-.44	.001
Rate of tension decline (g/min)	75	.38	.001
Rate of tension decline (g/g/min)	75	.35	.002
Rate of tension decline (%/min)	75	.33	.004
VENOUS BLOOD pO_2 (mm Hg) VS:			
Time from beginning of stimulation (min)	75	.28	.001
OXYGEN (A-V) DIFFERENCE (mm Hg) VS:			
Muscle tension development (kg)	75	.39	.001

TABLE IV SUMMARY OF SIGNIFICANT ($p < .01$) CORRELATIONS BETWEEN ARTERIOVENOUS AND TENSION-TIME PARAMETERS

PARAMETERS CORRELATED	NUMBER OF OBSERVATIONS	r	PROBABILITY THAT r=0
ARTERIAL HEMOGLOBIN CONCENTRATION (g%) VS:			
Arterial blood hematocrit (g%)	74	.97	.001
OXYGEN (A-V) DIFFERENCE (mm Hg) VS:			
Arterial blood pO_2 (mm Hg)	74	.83	.001
Lactate (A-V) difference (μM/g/min)	75	-.38	.001
Muscle oxygen uptake (μl/g/min)	74	.37	.001
Venous blood pCO_2 (mm Hg)	75	.30	.009
VENOUS BLOOD pH VS:			
Venous blood pCO_2 (mm Hg)	75	-.54	.001
Muscle blood flow (ml/g/min)	74	.34	.003
Lactate (A-V) difference (μM/g/min)	75	.31	.006
VENOUS BLOOD pCO_2 (mm Hg) VS:			
Diastolic blood pressure (mm Hg)	75	.45	.001
Lactate (A-V) difference (μM/g/min)	75	-.43	.001
Respiratory quotient	66	.37	.002
VENOUS BLOOD pO_2 (mm Hg) VS:			
Oxygen (A-V) difference (mm Hg)	75	-.52	.001
Muscle blood flow (ml/g/min)	74	.39	.001
MUSCLE OXYGEN UPTAKE (μl/g/min) VS:			
Muscle blood flow (ml/g/min)	74	.53	.001
Respiratory quotient (R.Q.)	66	-.32	.008
SYSTOLIC BLOOD PRESSURE (mm Hg) VS:			
Muscle blood flow (ml/g/min)	74	.33	.004
Diastolic blood pressure (mm Hg)	75	-.31	.008

TABLE V. SUMMARY OF SIGNIFICANT ($p < .01$) CORRELATIONS AMONG ARTERIOVENOUS PARAMETERS

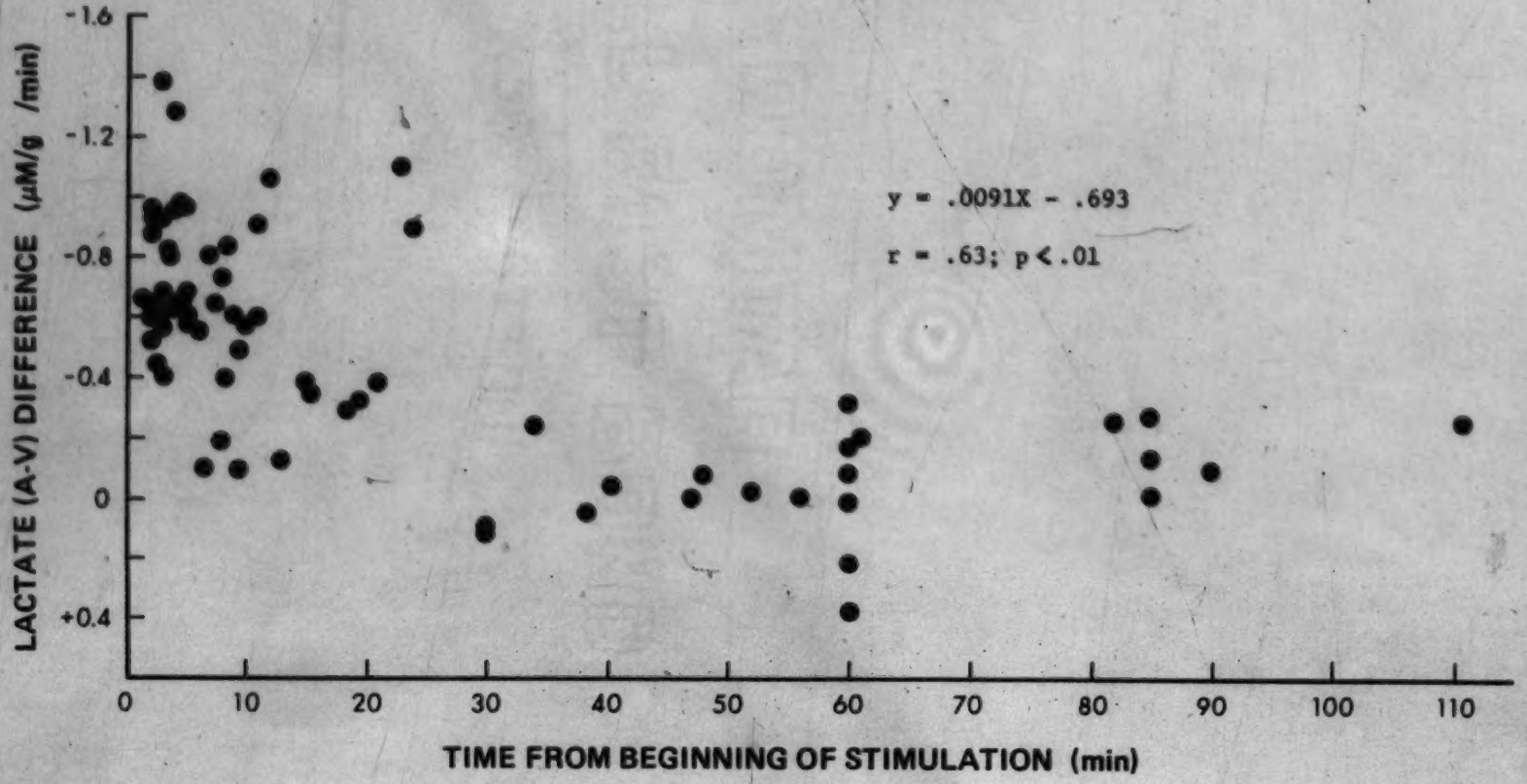


Figure 5: Relationship Between Lactate (A-V) Difference and Elapsed Time From Beginning of Stimulation

(mmHg) also showed a high degree of relationship ($r=.83$, $p<.01$) (Figure 6). This latter relatively high relationship occurred in spite of a lower but significant ($p<.01$) relationship between muscle O_2 uptake (ml/gm/min) and arteriovenous O_2 difference (mmHg) ($r=.37$, $p<.01$). This suggests the ability of the muscle to autoregulate blood supply to metabolic demands within the ranges of arterial pO_2 involved in the present study.

MUSCLE TISSUE CONCENTRATIONS

Muscle ATP and CP Concentration

The ATP and CP concentrations are summarized in Table VI. Single-factor analyses of variance performed on right control leg ATP and CP concentrations illustrated no significant differences ($p>.05$) among control muscle group means for either parameter.

Individual t-tests performed on right control vs. left stimulated ATP concentrations for each group illustrated significant differences ($p<.01$) in the 80% and 65% groups, and in the 50% and 50%L groups ($p<.05$). However, analysis of variance performed on the left stimulated muscle means showed no significant differences ($p>.05$).

Muscle CP concentrations were significantly lower ($p<.01$) in the left stimulated muscles when compared to their right controls in all tension groups, and no differences were present among the left muscle group means ($p>.05$).

Muscle Glycogen Concentration

The muscle glycogen data is summarized in Table VII.

Single factor analysis of variance indicated no significant differences among right control leg means ($p>.05$). Individual t-tests performed on right vs. left muscle glycogen concentrations for each group

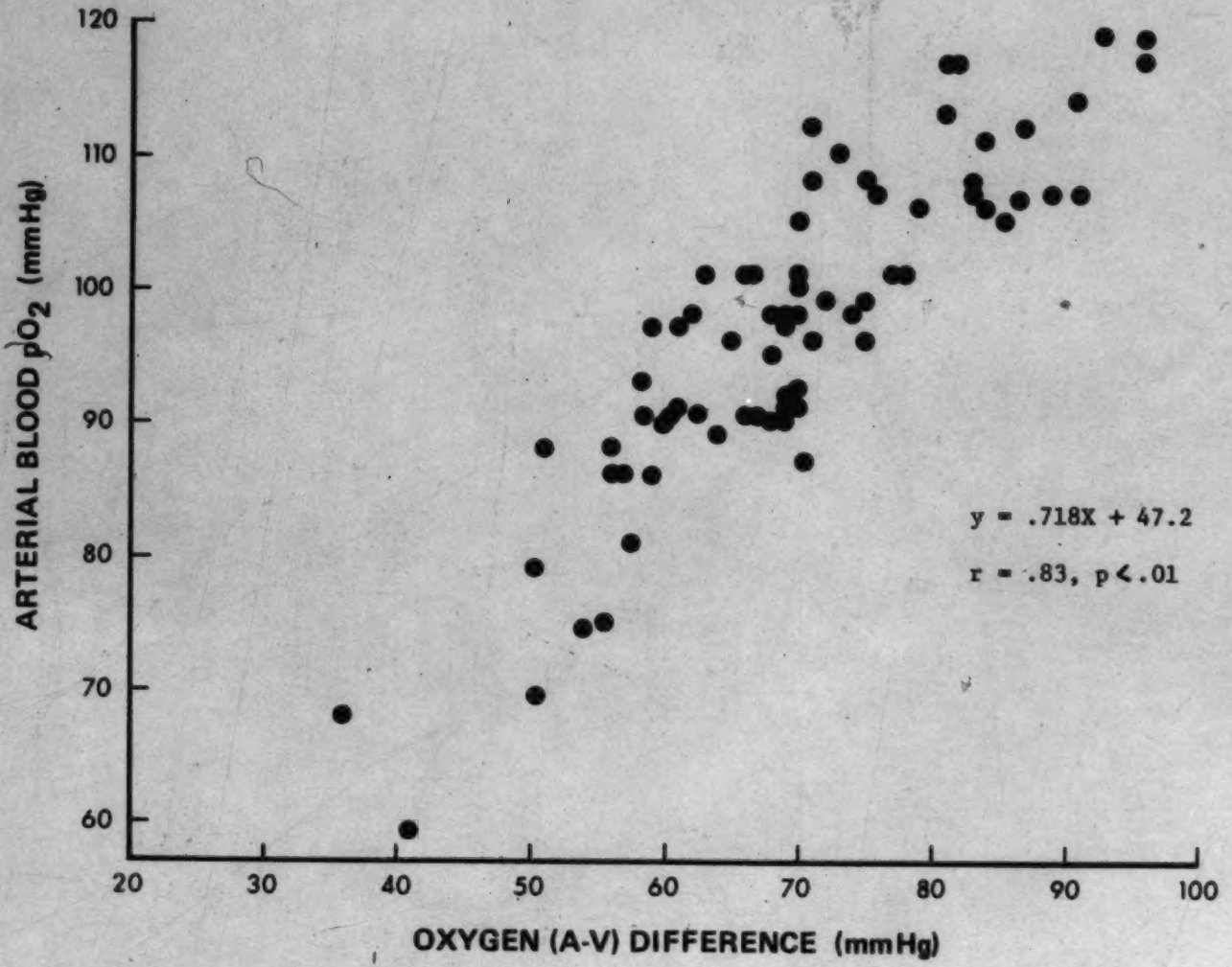


Figure 6: Relationship Between Arterial pO_2 and Oxygen (A-V) Difference

	ATP CONCENTRATION (uM/g)		CP CONCENTRATION (uM/g)	
	RIGHT	LEFT	RIGHT	LEFT
80%	4.01 ± 0.38	2.54 ± 0.20**	7.55 ± 0.58	3.58 ± 0.23**
65%	4.21 ± 0.22	2.34 ± 0.30**	8.29 ± 0.92	3.70 ± 0.25**
50%	4.38 ± 0.33	3.21 ± 0.40*	9.93 ± 1.16	6.15 ± 0.43**
50%L	3.63 ± 0.43	2.31 ± 0.42*	9.06 ± 0.83	5.28 ± 0.22**

TABLE VI MUSCLE ATP AND CP CONCENTRATION IN RIGHT CONTROL AND LEFT STIMULATED MUSCLES[a]

[a] Values are $\bar{X}$ ± SEM

* Significant difference ($p < .05$) between left stimulated and right control muscle

** Significant difference ($p < .01$) between left stimulated and right control muscle

GROUP	TOTAL MUSCLE GLYCOGEN CONCENTRATION (g%) RIGHT	LEFT	LEFT/RIGHT RATIO	TOTAL GLYCOGEN UTILIZED (g%)
80%	0.22 ± 0.05	0.15 ± 0.04	0.71 ± 0.05	0.07 ± 0.02
65%	0.16 ± 0.04	0.09 ± 0.02	0.62 ± 0.09	0.07 ± 0.02
50%	0.12 ± 0.03	0.06 ± 0.01*	0.59 ± 0.10	0.06 ± 0.02
50%L	0.16 ± 0.04	0.06 ± 0.02*	0.48 ± 0.09	0.10 ± 0.03

TABLE VII MUSCLE GLYCOGEN CONCENTRATIONS, GLYCOGEN RATIOS (L/R) AND TOTAL GLYCOGEN UTILIZED IN RIGHT CONTROL AND LEFT STIMULATED GASTROCNEMIUS MUSCLES[a]

[a] Values are $\bar{X} \pm$ SEM

* Significant difference ($p < .05$) between left stimulated and right control muscles

showed significantly lower glycogen values in the left muscles of the 50% and 50%L groups ($p < .05$). Analyses of variance performed on left muscle glycogen, glycogen (L/R) ratios and on total glycogen utilized revealed that the level of glycogen utilization among tension groups was not significantly different ($p > .05$).

Muscle Lactate Concentration

A summary of the muscle lactate concentration data is contained in Table VIII.

Analysis of variance revealed no significant differences ($p > .05$) among groups in right control muscle lactate concentration. Although there was a tendency for muscle lactate levels in the right control muscle to decrease with time, this trend was considered physiologically insignificant. This was supported by pilot studies which illustrated that muscle lactate levels in right versus left unstimulated muscle were similar, and within normal values reported elsewhere (Appendix B). Analysis using t-tests revealed significantly higher lactate concentrations than control values in the stimulated muscles of the 80% ($p < .01$) and 65% ($p < .05$) groups. No significant differences were evident between control and stimulated muscle lactate concentrations in the 50% or 50%L groups. This result was substantiated by analysis of variance performed on the left muscle lactate means, in that significant differences ($p < .01$) existed when comparing the 80% and 65% means with those of the 50% and 50%L groups.

Muscle Pyruvate Concentration

The muscle pyruvate concentration data is summarized in Table VIII.

Analysis of variance indicated that no significant differences ($p > .05$) existed among either the right control or the left stimulated muscle concentration means. T-tests performed on individual group data

	MUSCLE LACTATE CONCENTRATION (μM/g)		MUSCLE PYRUVATE CONCENTRATION (μM/g)	
	RIGHT	LEFT	RIGHT	LEFT
80%	1.53 ± 0.57	9.94 ± 1.41**	0.16 ± 0.03	0.28 ± 0.15
65%	1.01 ± 0.22	6.99 ± 2.26*	0.15 ± 0.02	0.16 ± 0.01
50%	0.81 ± 0.23	1.42 ± 0.49***	0.14 ± 0.02	0.21 ± 0.07
50%L	0.92 ± 0.22	1.17 ± 0.30***	0.16 ± 0.01	0.16 ± 0.02

TABLE VIII MUSCLE LACTATE AND PYRUVATE CONCENTRATION IN RIGHT CONTROL AND LEFT STIMULATED GASTROCNEMIUS MUSCLES[a]

[a] Values are $\bar{X} \pm$ SEM

* Significant difference ($p < .05$) between left stimulated and right control muscles

** Significant difference ($p < .01$) between left stimulated and right control muscles

*** Significant difference ($p < .01$) when compared to 80% and 65% left muscle mean

	CYTOPLASMIC [NAD+]/[NADH] RATIO		MITOCHONDRIAL [NAD+]/[NADH] RATIO	
	RIGHT	LEFT	RIGHT	LEFT
80%	1561 ± 603	237 ± 126*	23 ± 15	20 ± 11
65%	1592 ± 435	750 ± 596	31 ± 16	9 ± 2
50%	1913 ± 525	2049 ± 938	18 ± 7	36 ± 10
50%L	1328 ± 182	1448 ± 561	46 ± 30	16 ± 4

TABLE IX ESTIMATED CYTOPLASMIC AND MITOCHONDRIAL [NAD+]/[NADH] RATIOS IN RIGHT CONTROL AND LEFT STIMULATED GASTROCNEMIUS MUSCLES[a]

[a] Values are $\bar{X} \pm$ SEM

* Significant difference ($p < .05$) between left stimulated and right control muscles.

showed no differences ($p > .05$) between left and right means in any of the tension groups.

Cytoplasmic $[NAD^+]/[NADH]$

Table IX contains the means for the estimated cytoplasmic $[NAD^+]/[NADH]$ ratio.

No significant differences ($p > .05$) were shown to exist among control muscle or stimulated muscle means using analysis of variance. Individual t-tests performed on the data for each tension group illustrated a significantly lower ($p < .05$) ratio in the left muscles of the 80% group as compared to the right control ratio. Differences between control and stimulated muscle ratios in all other tension groups were not significant ($p > .05$).

Mitochondrial $[NAD^+]/[NADH]$

A summary of the estimated $[NAD^+]/[NADH]$ data is included in Table IX.

Analysis of variance on control and stimulated muscle means, and t-tests performed on control vs. stimulated muscle means for each tension group, indicated no significant differences ($p > .05$).

Muscle Fibre Composition and Recruitment

Table X contains a summary of data resulting from the classification of fibre composition and recruitment in the gastrocnemius-flexor muscle group. Muscle fibres were classified as fast or slow twitch based on a positive (fast) or negative (slow) response to the myosin ATPase stain, as outlined by Peter *et al.* (1972).

All slow twitch fibres stained darkly and evenly on the NADH-diaphorase stain, attesting to the high oxidative potential of these fibres (see Appendix F). The slow twitch fibres in the control muscles also elicited the lightest relative staining intensity on the PAS stain

SAMPLE	NUMBER OF SAMPLES	NUMBER OF FIBRES COUNTED PER MICROGRAPH	TOTAL FIBRES COUNTED	% FAST TWITCH FIBRES
Right Gastrocnemius	22	157 ± 8	3451	51.7 ± 2.2
Left Gastrocnemius	22	149 ± 8	3277	52.3 ± 2.1
Total	44	153 ± 5	6728	52.0 ± 1.5

TABLE X FIBRE COMPOSITION OF GASTROCNEMIUS MUSCLE (MEDIAL HEAD)[a]

[a] Values are $\bar{X}$ ± SEM

for glycogen content.

The fast twitch fibres, however, showed some variation in the NADH diaphorase and PAS stains. The vast majority of these fibres stained moderately on the NADH diaphorase stain, eliciting the subsarcolemmal depositing of formazen granules characteristic of the fast-oxidative-glycolytic (FOG) fibre as described by Peter et al. (1972). However, there were fast twitch fibres which stained as darkly on the oxidative stain and as lightly on the PAS stain as the slow twitch fibres. In spite of the temptation to classify these latter fibres as FOG fibres, and the former as FG fibres, the decision was made to merely classify all fibres on the basis of twitch characteristics based on the myosin ATPase stain, and regard all fibres as having relatively high oxidative potential.

The fibre composition of the gastrocnemius muscle (medial head) is outlined in Table X. Pilot data relative to fibre composition of both the lateral head and flexor component of the muscle group illustrated that the medial head was representative of the entire muscle group. It was also deemed necessary to utilize the fibre data from the medial head as this was also the site of the freeze-clamped samples.

Analysis using t-tests revealed no significant differences ($p > .05$) between the fibre proportions in left and right medial gastrocnemius.

Subjective evaluation of fibre recruitment based on PAS staining intensity suggested a recruitment of all fibres with electrical stimulation (see Appendix F), due to the fact there was a decrease in the staining intensity in all fibres compared to the non-stimulated control muscle. In the 50% group muscles, all fast twitch fibres showed either a very light or a negative staining intensity on the PAS stain, with the slow twitch fibres staining darker than the fast twitch

fibres, but lighter than the slow fibres in the corresponding control muscle.

The 50%L fibre profile suggested a continued recruitment of the slow fibres with time, as witnessed by a further slight decrease in the general staining intensity of these fibres compared to the 50% group. No statistical analysis was performed on the data.

Correlations Between Muscle Metabolite Concentrations and Tension-Time Parameters

Table XI includes all those correlations which indicated a significant relationship ($p < .01$). The complete correlation matrix is included in Appendix E.

The highest single correlation was between the rate of tension decline (g/min) and muscle lactate concentration ($r=.80$, $p < .001$). This relationship is illustrated in Figure 7. The three highest correlation coefficients involved relationships between muscle lactate concentration and rate of tension decrement (Table XI).

Comparison of 65%L and 50%L Groups

Table XII contains a summary of means for selected parameters measured during the period of "steady state" in tension development in the 65%L and 50%L groups. Measurements indicated were taken during the last 5 minutes of stimulation. No significant differences ($p < .05$) were noted between the 65%L and 50%L group means, suggesting that these parameters did not significantly influence the tension at which these muscles levelled off.

A summary of arteriovenous dynamics as measured during the steady-state period of tension development in the two level-off groups is included in Table XIII. No significant differences ($p < .05$) in any of the arteriovenous parameters were evident, as indicated by statistical analysis using t-tests.

PARAMETERS CORRELATED	NUMBER OF OBSERVATIONS	r	PROBABILITY THAT r=0
LEFT MUSCLE LACTATE CONCENTRATION (uM/g/min) VS:			
Rate of tension decline (g/min)	28	.80	.001
Rate of tension decline (g/g/min)	28	.78	.001
Rate of tension decline (%/min)	28	.76	.001
Muscle tension development at sample (kg)	28	.63	.001
Time from beginning of stimulation (min)	28	-.63	.001
MUSCLE TENSION DEVELOPMENT AT SAMPLE (% OF ORIGINAL) VS:			
Left muscle CP concentration (μM/g)	23	-.63	.001
Left muscle glycogen concentration (g%)	29	.52	.004
MUSCLE TENSION DEVELOPMENT AT SAMPLE (KG) VS:			
Right muscle weight (g)	26	.66	.001
Left muscle weight (g)	25	.62	.001
Muscle glycogen concentration (g%)	29	.57	.001

TABLE XI SUMMARY OF SIGNIFICANT ($p < .01$) CORRELATIONS BETWEEN MUSCLE METABOLITE CONCENTRATIONS AND TENSION-TIME PARAMETERS

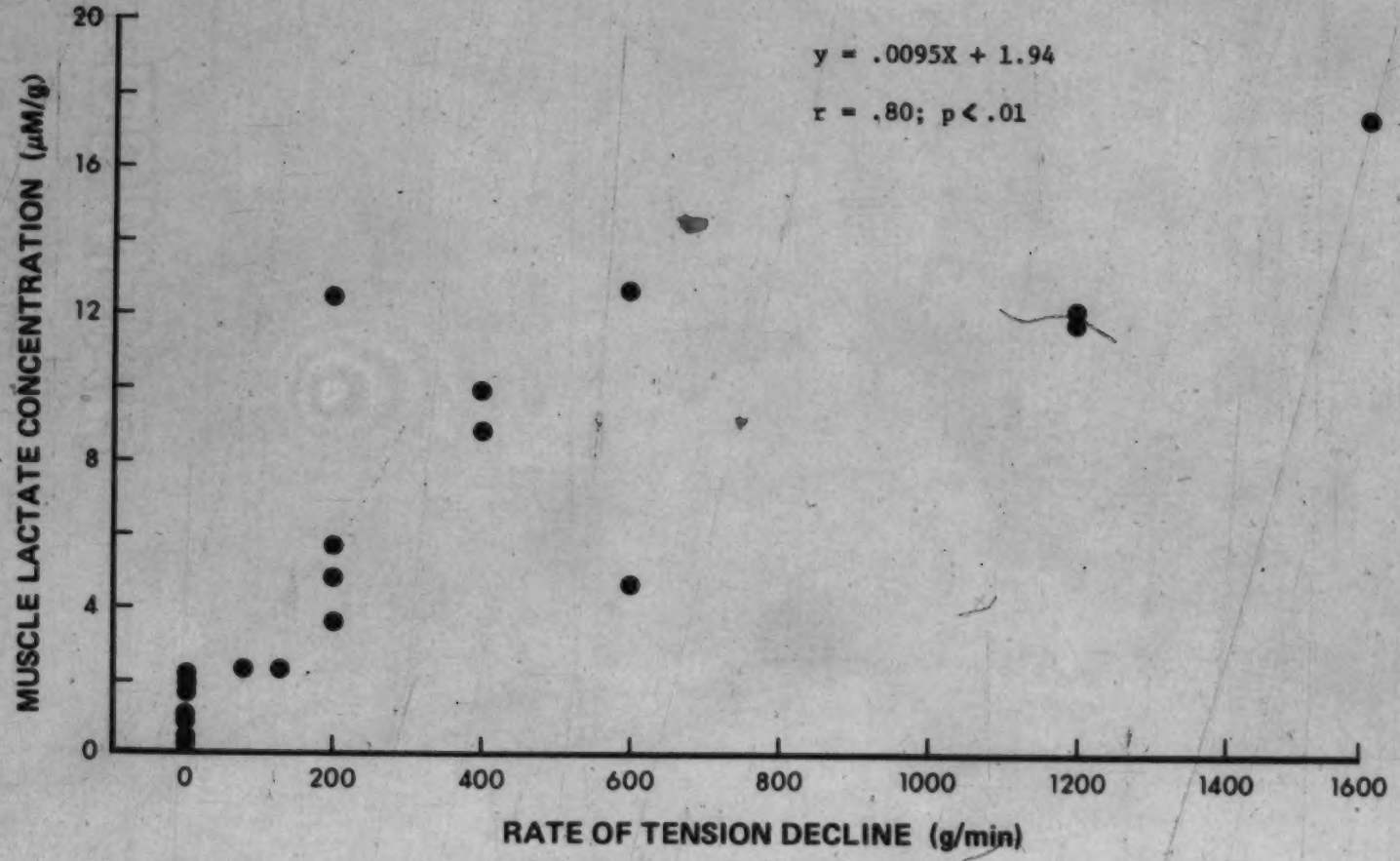


Figure 7: Relationship Between Muscle Lactate Concentration and Rate of Tension Decline at Sample Time

PARAMETER	GROUP 50%L	65%L
Arterial blood pO_2 (mm Hg)	91.9 ± 2.9	94.2 ± 6.1
Arterial blood pCO_2 (mm Hg)	39.3 ± 3.1	39.2 ± 2.5
Arterial blood pH	7.23 ± 0.02	7.25 ± 0.02
Arterial blood hemoglobin concentration (g%)	12.8 ± 1.0	12.8 ± 0.8
Arterial blood hematocrit (%)	38.0 ± 3.2	37.3 ± 2.3
Systolic blood pressure (mm Hg)	98 ± 6	92 ± 3
Diastolic blood pressure (mm Hg)	40 ± 4	51 ± 5
Muscle compartment temperature (°C)	39.3 ± 0.5	40.0 ± 0.6

TABLE XII COMPARISONS OF SELECTED PARAMETERS MEASURED DURING THE LEVEL-OFF PERIOD IN THE 65%L AND 50%L GROUPS[a]

[a] Values are $\bar{X}$ ± SEM

PARAMETER	LEVEL-OFF GROUP 65%L	50%L
Muscle oxygen uptake (ul/g/min)	72.2 ± 4.5	58.3 ± 6.2
Muscle blood flow (ul/g/min)	0.94 ± 0.16	0.64 ± 0.07
Lactate (A-V) difference (uM/g/min)	0.05 ± 0.20	-0.07 ± 0.05
Glucose (A-V) difference (uM/g/min)	0.14 ± 0.17	0.15 ± 0.20
Water (A-V) difference (uM/g/min)	22.1 ± 8.9	5.0 ± 8.4
FFA (A-V) difference (neq/g/min)	47.4 ± 32.6	8.8 ± 71.3
Respiratory quotient (R.Q.)	0.92 ± 0.19	0.80 ± 0.12

TABLE XIII ARTERIOVENOUS DYNAMICS MEASURED FIVE MINUTES PRIOR TO THE END OF THE LEVEL-OFF PERIOD IN 65%L AND 50%L GROUPS[a]

[a] Values are $\bar{X} \pm$ SEM

The data in Table XIV summarize the muscle metabolite profiles in the 65%L and 50%L groups. T-tests performed on left vs. right concentrations of muscle glycogen, muscle ATP and muscle CP concentrations showed similar significant decreases ($p < .05$) in the left muscles for both groups. The CP concentration decrease in the 50%L group was significant ($p < .01$). Muscle lactate concentration was significantly lower ($p < .05$) in the left as opposed to the right muscle of the 65%L group, while no difference ($p > .05$) existed between the left and right muscle means in the 50%L group. The t-tests performed separately on the right control muscle concentrations (65%L vs. 50%L) and on the left stimulated muscle concentrations (65%L vs. 50%L) indicated no significant differences ($p > .05$) between pairs of means.

Table XV contains a list of the highest correlation coefficients found between specific arteriovenous parameters or muscle parameters, and the percent of original tension at which the muscles in the 65%L and the 50%L groups levelled off. The highest single correlation, and the only one showing a significant relationship ($p < .01$) was that relating percent of original tension at which level-off occurred and the lactate (A-V) difference measured at 65% of original tension ($r=.82$, $p=.003$)(Figure 8). All other correlation coefficients were lower and not significant ($p > .05$).

Examination of fibre recruitment patterns in the 65%L and 50%L groups (see Appendix F) suggested a slight difference in fibre glycogen utilization in these groups. In the 50%L group the fast twitch fibres appeared to be devoid of glycogen, as witnessed by a negative reaction to the PAS stain, with the slow twitch fibres staining either very lightly or, in some cases, negatively. Examination of the 65%L muscle

PARAMETER	GROUP 65%L RIGHT	65%L LEFT	50%L RIGHT	50%L LEFT
Muscle glycogen concentration (g%)	0.18 ± 0.03	0.08 ± 0.02*	0.16 ± 0.04	0.06 ± 0.02*
Muscle glycogen (L/R)	---	0.49 ± 0.05	---	0.48 ± 0.09
Muscle glycogen utilized (g%)	---	0.10 ± 0.03	---	0.10 ± 0.03
Muscle ATP concentration (μM/g)	4.65 ± 0.31	3.19 ± 0.44*	3.63 ± 0.43	2.31 ± 0.42*
Muscle CP concentration (μM/g)	8.77 ± 1.42	5.26 ± 0.52*	9.06 ± 0.90	5.28 ± 0.22**
Muscle lactate concentration (μM/g)	1.71 ± 0.30	0.59 ± 0.22*	0.92 ± 0.22	1.17 ± 30
Muscle pyruvate concentration (μM/g)	0.15 ± 0.03	0.14 ± 0.03	0.16 ± 0.01	0.16 ± 0.02
Cytoplasmic [NAD+]/[NADH]	962 ± 348	3186 ± 1296	1328 ± 182	1448 ± 561
Mitochondrial [NAD+]/[NADH]	17 ± 5	97 ± 74	46 ± 30	16 ± 4
Fast twitch fibres (%)	---	53 ± 5	---	58 ± 3
Slow twitch fibres (%)	---	47 ± 3	---	42 ± 3

TABLE XIV MUSCLE METABOLITE LEVELS IN THE LEFT AND RIGHT GASTROCNEMIUS MUSCLE IN 65%L and 50%L GROUPS[a]

[a] Values are $\bar{X}$ ± SEM

* Significantly different from right muscle ($p < .05$)

** Significantly different from right muscle ($p < .01$)

PARAMETER	NUMBER OF OBSERVATIONS	r	PROBABILITY THAT r=0
Lactate (A-V) difference (uM/g/min) measured at 65%	10	.82	.003
Muscle blood flow (ml/g/min) measured at 65%	10	.57	.084
Plasma water (A-V) difference (ul/g/min) measured at level-off	10	.57	.054
Muscle lactate concentration (uM/mg)	11	-.52	.099
Muscle blood flow (ml/g/min) measured at level-off	12	.50	.097

TABLE XV RELATIONSHIPS OF SELECTED PARAMETERS TO TENSION AT WHICH LEVEL-OFF OCCURRED (% OF ORIGINAL) DURING CONTRACTIONS

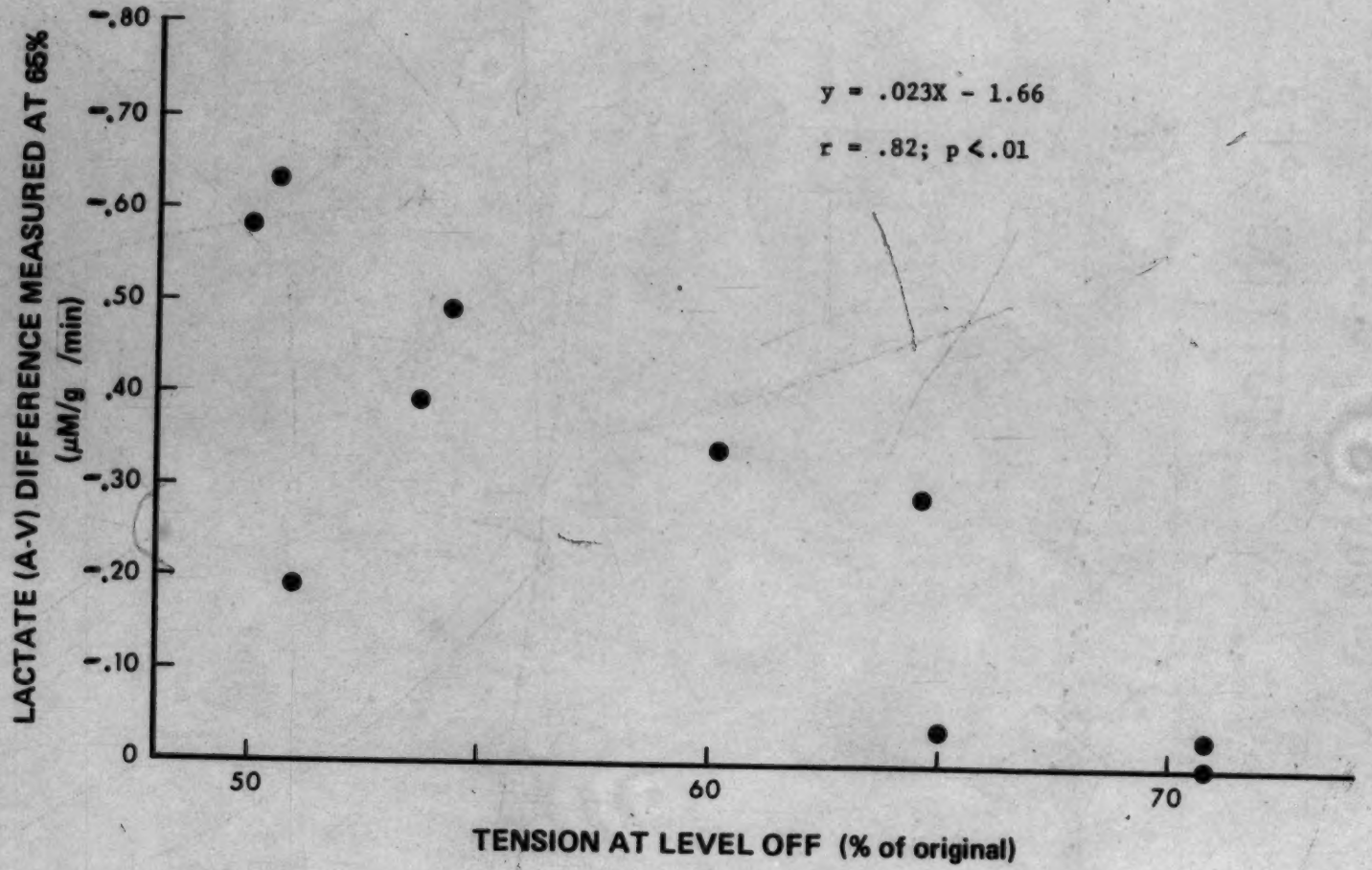


Figure 8: Relationship Between Lactate (A-V) Difference Measured at 65% and Tension at Which Level Off Occurred

sections revealed fast twitch fibres which stained negatively, for the most part, although evidence of small amounts of glycogen was present due to lightly staining fast twitch fibres. All slow twitch fibres tended to contain relatively high amounts of glycogen as witnessed by a darker staining intensity in these fibres compared to the slow twitch fibres of the 50%L muscles (Appendix F).

DISCUSSION

The progressive reversible decrement in the ability of electrically stimulated or voluntarily recruited muscle to maintain high levels of tension output is well documented (Stainsby and Welch, 1966; Welch and Stainsby, 1967; Hirche et al., 1970; Hirche et al., 1971; Simonson, 1971; Baldwin and Tipton, 1972; Fitts et al., 1973; Fitch et al., 1975; Morganroth et al., 1975). The pattern of this decrement is dependent upon whether contractions are sustained or rhythmic (Simonson, 1971; Stainsby and Welch, 1966; Hirche et al., 1975), the muscle fibre composition (Baldwin and Tipton, 1972), and the rate of stimulation of the muscle (Stainsby and Welch, 1966; Welch et al., 1974). The tension decline patterns of the working muscles witnessed in the present investigation are similar to those reported by other investigators using in situ muscle preparations (Welch and Stainsby, 1967; Hirche et al., 1970; Hirche et al., 1971; Baldwin and Tipton, 1972; Fitts et al., 1973; Morganroth et al., 1975), demonstrating an initial rapid decline in tension followed by a progressively slower decrease to an apparent steady state in tension development. This pattern has been illustrated in voluntary contractions (Simonson, 1971), and it has been illustrated that humans can perform isometric contractions from 15 to 50 per cent of maximum voluntary contraction, for periods up to 90 minutes when contractions are of short duration, rhythmic, and interspersed with short rest intervals (Gollnick et al., 1974). This pattern is comparable to the level-off phenomenon witnessed in the present investigation.

Maintenance of Viability of the Muscle Preparation

An attempt was made in these experiments to outline metabolic correlates to tension decline in a maximally stimulated in situ muscle

preparation. This necessitated the use of a preparation which could be maintained at a consistent degree of viability throughout the experiment. Any physiological deterioration on the part of the anesthetized animal or the muscle preparation itself might potentially affect the metabolic and contractile properties of the working muscle, thereby limiting any conclusions as to intramuscular metabolic roles in tension decline during continuous contractions. For this reason, methodological attempts were made at preventing such deterioration from occurring.

Of prime concern was the role played by the stimulated nerve trunk and at the neuromuscular junction, in the gradual tension decline of the working muscle group. Results from previous research involving *in situ* muscle (Hirche *et al.*, 1970; Stainsby, 1970) as well as human muscle in vivo have eliminated the neuromuscular junction as a possible site of fatigue during indirect stimulation, unless the nerve trunk stimulation was uninterrupted, at a tetanizing frequency, for 15 to 60 minutes (Simonson, 1971). Although stimulation of the severed nerve trunk has been shown to elicit muscle tension decrement curves comparable to those witnessed when using direct muscle belly stimulation (Stainsby, 1970; Hirche *et al.*, 1970), this was not the case in the pilot experiments conducted prior to the experimental series in this investigation. Changing immediately from nerve trunk stimulation to muscle belly stimulation during continuous contractions resulted in a 3 to 4 fold increase in muscle tension development. The reason for the lack of similarity in tensions elicited via indirect and direct muscle stimulation is unexplained, but may have been due to inadequate maintenance of nerve trunk viability, incomplete stimulation of the entire nerve trunk, neuromuscular failure, or a combination of these factors.

For these reasons, direct electrical stimulation delivered to the muscle belly was utilized throughout the experimental series.

In the level-off experiments, which constituted the longest experiments in the series, selected parameters were measured to give indications as to the physiological constancy of the preparation. Rectal temperature, arterial blood glucose concentration, hemoglobin, and arterial pO_2 remained unchanged during the prolonged experimental manipulation (Appendix E). However, there were significant decreases in both systolic and diastolic pressure, which reflected the gradual loss in blood volume as a result of surgery, and perhaps some decrease in arteriolar and venomotor tone with time under anesthesia. However, no significant relationships ($p > .05$) were evident between either of the pressures and individual tension parameters (Appendix E). In addition, the significant changes in arterial blood pCO_2, pH, hematocrit, lactate and free fatty acids could not account for differences in level-off tension in these experiments (Tables XII and XIII), and can be attributed most likely to a dilution effect of the intravenous Ringer's drip, and altered respiratory patterns during anesthesia.

The maintenance of the ability of the muscle to autoregulate its own blood supply to metabolic demand, in spite of denervation, is a well known index of muscle preparation viability (Stainsby and Otis, 1964; Duran and Renkin, 1974). Maintenance of autoregulatory ability of the muscle preparations used in the present study was suggested by the relatively low correlation ($r=.24$; $p=.04$) between arterial pO_2 and muscle oxygen uptake, compared to the high degree of relationship ($r=-.88$; $p < .01$) between arterial pO_2 and (A-V) O_2 difference (Figure 6). Thus, although arterial pO_2 dictated to a large degree the extent of

extraction of oxygen from arterial blood, this influence did not appear to significantly affect the oxygen consumption of the muscle during contractions, probably due to the ability of muscle to increase its blood supply relative to metabolic demand. This interpretation is consistent with the research of Stowe et al. (1975), who attributed the local control of working muscle blood flow to altered pO_2 and pH of the effluent blood and their effects, either independent or combined, on vascular smooth muscle tone, and therefore, resistance to flow. This autoregulatory ability of denervated muscle has been illustrated by Stainsby and Otis (1964), who demonstrated in a similar dog gastrocnemius muscle preparation, that muscle oxygen uptake was maintained constant in the contracting muscle, in spite of arterial pO_2 levels below normal, until a critical arterial pO_2 of 40 mmHg was reached. In no case in the currently discussed series of experiments was the arterial pO_2 during contractions below 60 mmHg (Figure 6).

Recently, Barclay and Stainsby (1975) illustrated in isolated canine gastrocnemius muscle that increasing spontaneous blood flow through the muscle group while it was contracting at a steady level of tension development resulted in an increase in both tension development and oxygen uptake. Their calculations indicated that the resulting increase in oxygen delivery to the working muscle was of the same magnitude as the increased oxygen delivery resulting from arterial pO_2 elevation, which resulted in no change in tension development or oxygen uptake (Stainsby et al., 1972). Thus, of the two conditions in which the rate of oxygen delivery to the muscle was enhanced, the artificial increase in muscle blood flow caused increased muscle oxygen consumption and contractile capabilities, while the artificial increase in arterial oxygen content did not. The conclusion

of Stainsby et al. (1972) was that muscle tension and oxygen uptake are dependent upon muscle blood flow rate, and that the mechanism does not involve the rate of oxygen delivery. Although the ramifications of these findings to the present study are not entirely clear, the results from the current series of experiments suggested no significant effect of pressure alterations on tension decrement in any of the experiments (Appendix E) or in the tension at which level off occurred in the level off groups (Table XIII). In addition, although significant correlations existed between muscle blood flow and selected tension indices (Table IV), the correlations were not as high as those involving metabolic parameters to be discussed.

Muscle Metabolic Rate During Contractions

The resting oxygen consumption and blood flow measurements taken during the prestimulation period are consistent with previous reports using similar preparations (Stainsby and Otis, 1964; DiPrampero et al., 1968; Piiper et al., 1968; Hirche et al., 1971). Although the maximum oxygen uptake capability of each working muscle was not directly measured, indirect evidence suggests that the stimulation pattern elicited a muscle metabolic rate which might be considered below maximum aerobic capacity. The highest mean oxygen consumption of the working muscle of 84.0 ± 9.0 µl/g/min demonstrated in the present investigation is below the maximum values which have been reported elsewhere. Stainsby and Welch (1966) reported that the oxygen uptake of the working dog gastrocnemius muscle group performing twitch contractions reached a maximum level of approximately 30 times rest, which is well over the approximately 12 fold increase reported herein. Absolute oxygen uptake levels over 100 µl/g/min (Welch and Stainsby, 1967;

Piiper et al., 1968; Hirche et al., 1971; Stainsby and Barclay, 1972; Hirche et al., 1975) are generally accepted as maximum for this muscle group, during both twitch (Welch and Stainsby, 1967; Piiper et al., 1968) and tetanic (Hirche et al., 1971; Stainsby and Barclay, 1972) types of rhythmic contractions. In addition, reported muscle blood flows at these high metabolic rates have been in excess of those reported in this study (Piiper et al., 1968; DiPrampero et al., 1969; Hirche et al., 1975). Further evidence suggesting the muscle was working at a submaximal metabolic rate is evident upon examination of the arterial and venous pO_2 dynamics during the contraction period. Stainsby and Otis (1964) have reported that the critical venous pO_2 draining the working muscle is approximately 10 mmHg, below which there are indications of decreases in developed tension and oxygen consumption due to insufficient oxygen availability. Measurements of venous pO_2 values during the contraction period in this investigation illustrated mean pO_2 levels well above this critical level (Table II). In addition, arterial pO_2 values during contractions in all experiments were maintained close to normal unanesthetized levels and in no instance fell below 60 mmHg (Figure 6), which is well above the critical arterial pO_2 of 40 mmHg below which oxygen becomes limiting during contractions (Stainsby and Otis, 1964).

Although significant increases in resistance to muscle blood flow have been reported for this muscle group when performing sustained isometric contractions, Hirche et al. (1970) have illustrated that during rhythmic isometric contractions similar to the type utilized in this investigation, there is no significant resistance to flow. In fact, Hirche et al. (1970) claim that flow is facilitated by the compression of

veins during contractions. Had flow limited oxygen consumption in this series of experiments, it would have been shown by an approach of venous pO_2 levels to the critical level during periods of high tension decrement (80% sampling interval), and by a strong relationship between flow and oxygen uptake. A significant, although low, relationship appeared to exist between blood flow and oxygen consumption ($r=.53$, $p < .01$), a phenomenon which has been illustrated by Barclay and Stainsby (1975) in this muscle group when contracting at a steady state of tension production. This relationship, however, appeared to be independent of tension development indices, as illustrated by the relationships of blood flow and oxygen consumption to tension indices (Appendix E).

The maximum mean muscle lactate concentration reported in this investigation (9.94 ± 1.41 μM/g in 80% group) is below the levels reported by Hirche et al. (1973, 1975), which approached 17.5 ± 2.1 μM/g after 2 minutes of contraction. These data, in addition to the comparative oxygen consumption, blood flow and blood pO_2 results previously outlined, strongly suggest that the muscles in the present study were working at a metabolic rate below maximum capacity, in the presence of sufficient quantities of oxygen, at least at the 80% sampling interval and thereafter.

Arteriovenous Parameters and Tension Decrement

The data reported on the arteriovenous dynamics have been corrected for alterations in plasma water content of the blood passing through the muscle, using the formulae of Schlein et al. (1973) (Appendix D). Therefore, the arteriovenous results cannot be directly compared to those reported by other investigators who have used the Fick principle

(Rushmer, 1970) without this correction, although trends should be comparable.

By using this formula, it was possible to estimate the uptake and release of water by the working muscles. The uptake of plasma water of 19.9 ± 5.4 µl/g/min is lower in magnitude than the maximum level of 33.9 ± 8.0 µl/g/min reported by Schlein et al. (1973) in their working dog sartorius muscles. This may possibly be due to higher metabolic rates than those found in this investigation, although these were not reported by Schlein et al. Increases in intracellular and interstitial water content have been reported in human quadriceps muscles during heavy bicycle ergometer work (Bergstrom et al., 1971). Jacobsson and Kjellmer (1964) suggested that this phenomenon of water uptake by working muscle is due to the osmotic gradient generated by the addition of lactate to the intracellular and interstitial spaces, thus causing water movement from plasma into the muscle. In spite of the tendency of the water arteriovenous dynamics during contractions to follow the tension decline pattern, the highest individual correlation relating (A-V) water difference to any tension parameter was low compared to other metabolic factors ($r=.27$; $p=.02$) between arteriovenous water difference and tension in kg at sample time, see Appendix E). It is proposed that the plasma water influx during the initial periods of high tension decrement was merely a consequence of other metabolic and/or cardiovascular changes during contractions which were themselves more closely related to tension decline.

There were no significant relationships between arteriovenous water differences and arteriovenous lactate difference measured at the same time (Appendix E, Table XIX). This does not preclude the influx of

plasma water into contracting muscles due to lactate accumulation, as it has been shown that lactate may accumulate in working muscle at the beginning of contraction, and not be reflected in magnitude via (A-V) lactate differences (Hirche et al., 1973). Since water arteriovenous difference and muscle lactate concentration were not measured less than 5 minutes apart, it was impossible to estimate this relationship. Therefore, water influx during contractions may have been due to the osmotic effect suggested by Jacobsson and Kjellmer (1964), and may also have been facilitated by an increase in capillary pressure during contractions, which has been shown to result in muscle edema in contracting canine gastrocnemius (Hirche et al., 1970). Whatever the reason for the arteriovenous water dynamics witnessed in this investigation, they cannot be proposed as being significant in tension decline in the working muscles.

The muscle lactate output measured during the prestimulation resting period is within the range of values which have been reported by other investigators using a similar preparation (Stainsby and Welch, 1966; Hirche et al., 1971; Hirche et all, 1975). The highest working muscle lactate output was recorded during the initial 80% sampling interval, after which lactate output gradually declined to resting levels (Table III). The absolute arteriovenous difference of -0.72 $\pm$ 0.07μM/g/min recorded at 80% is below the lactate output levels of above 1.0μM/g/min (Welch and Stainsby, 1967; Hirche et al., 1973a) and as high as 3.0μM/g/min (Hirche et al., 1975) reported for similar preparations working at higher metabolic rates. The fact that lactate (A-V) difference, unlike any other single arteriovenous parameter, was significantly correlated with all tension-time indices (Table IV),

suggests a possible role of lactate production in muscle tension decrement. Although correlational data does not infer cause and effect, the relationships between arteriovenous lactate and tension indices strongly suggest that lactate may be involved either as a direct affector or as an indicator of another factor which is involved in influencing tension. The single highest correlation was between lactate (A-V) difference and time ($r=.63$, $p<.01$). It thus appears that the accumulation of lactate in muscle tissue during the initial phases of contraction, and failure of the circulatory system to remove it as quickly as it was being produced, may have taken place. This phenomenon of insufficient removal of muscle-produced lactate has been attributed to the muscle membrane constituting a barrier to the non-ionized form of lactic acid (Hirche et al., 1975). Such a limitation to lactate efflux from muscle during periods of high lactate production could result in a time-related phenomenon, with lactate (A-V) dynamics failing to reflect muscle metabolic status at any given point in time. This relationship is suggested by the present results (Table IV).

The slight glucose uptake (Table III) by the resting muscle found in the present investigation has been reported elsewhere (Chapler and Stainsby, 1968; DiPrampero et al., 1969; Hirche et al., 1970; Costin et al., 1971; Schlein et al., 1973; Chapler and Katrusiak, 1974). The negative glucose arteriovenous difference witnessed at the 80% sampling interval (-0.03 ± 0.09 μM/g/min), suggesting a tendency towards glucose output during this initial period of tension decline, has also been shown by Corsi et al. (1969) in tetanized muscle. Their reported glucose output levels during the initial period of contraction (30 sec into the contraction period) were in some cases more than twice the

mean value obtained in the present investigation. Similarly, Bergstrom and Hultman (1966) found that there was no positive (A-V) difference in glucose across the legs of humans working on the bicycle ergometer, thereby suggesting a tendency towards glucose release, or nonutilization.

More recently, Schlein et al. (1973) have illustrated glucose uptake by contracting muscles when a correction is made for plasma water uptake by muscle. The standard Fick principle without this correction erroneously indicated a net glucose release from the muscle. The present investigation is the first to illustrate a tendence towards glucose release by contracting muscle early in the contraction period, even when the correction is made for the increased glucose concentration of venous blood due to plasma water flux into the muscle. This glucose output appears to coincide with increased levels of glucose-6-phosphate in working muscle (Corsi et al., 1969; Hultman and Bergstrom, 1973) during periods of high glycogenolysis (Corsi et al., 1969; Hultman and Bergstrom, 1973). Indeed, Hultman and Bergstrom (1973) have measured increased levels of free glucose in the quadriceps muscles of humans performing heavy bicycle ergometer work. They attribute this to the fact that glucose produced by the action of 1,6-amyloglucosidase may not be phosphorylated at a rate concomitant with its production.

After this initial output, glucose uptake increased although not significantly, to a level of 0.35 $\pm$ 0.15 μM/g/min by 60%, which is higher than the maximum values that have been reported in muscles performing twitch contractions of 1 to 4 per second (Chapler and Stainsby, 1967; Costin et al., 1971) and is comparable to that reported at twitch rates of 5 per second, and during high intensity rhythmic tetanic contractions (Chapler and Stainsby, 1968; Hirche et al., 1970; Chapler

and Katrusiak, 1974). However, the degree of variation in the glucose data was large, and failed to illustrate significant relationships with any tension decline or time indices, thus eliminating it as a possible limiting factor itself, or indication of any limiting factor related to its respective uptake, metabolism or release.

The only comparable result in the arteriovenous FFA data was an output of FFA by muscle at rest. This phenomenon has been reported by previous investigators (DiPrampero et al, 1969; Keul et al., 1974). During the contraction period, there appeared to be no consistent trend relative to FFA uptake or release and the variability was extremely high. Similarly, the R.Q. estimates, although indicating a trend towards fat metabolism toward the end of the contraction periods in the prolonged experiments, showed a large amount of inter-animal variation. Consequently, no discernible relationships of either arteriovenous FFA or R.Q. with tension-time indices were demonstrated.

Muscle Tissue Concentrations and Tension Decrement

Measured muscle lactate concentrations during contractions (Table VIII) were lower than those reported in similar preparations (Hirche et al., 1973a; Hirche et al., 1975), in stimulated rat gastrocnemius muscle (Edington et al., 1973), and in the quadriceps muscle of humans performing at 75% of maximum aerobic capacity (Karlsson et al. 1971). During the working period muscle lactate concentration was more highly related to tension decrement indices than any other single metabolic parameter (Table XI, Figure 7). This result, in conjunction with the arteriovenous lactate data, support the suggestion that lactate accumulated in the muscle during the initial period of contraction, either constituted, or was in some way intimately related to, a factor

limiting the ability of the muscle to maintain a high degree of work output. A limiting aspect of muscle lactate accumulation, mediated via a concomitant increase in intramuscular hydrogen ion concentration, has been proposed by a number of investigators (Karlsson et al., 1970; Karlsson et al., 1972; Hermansen et al., 1972). Katz (1970), on the basis of research on acidotic heart muscle, proposed the concept that the negative inotropic effect of acidosis may reflect a decrease in the number of active actin-myosin interactions due to displacement of troponin-bound calcium ion by hydrogen ion. Fuchs et al. (1970) investigated this possibility in vitro by examining the binding capabilities of isolated rabbit skeletal muscle troponin and calcium, which decreased at pH levels near those reported in the muscle of exercised humans (Hermansen et al., 1972). Recently, Hirche et al. (1975) have presented data which further support this concept. In their investigation, the pH of the arterial blood supplying the working gastrocnemius muscle preparation was altered by infusion of either sodium bicarbonate or hydrochloric acid. During the work period, both conditions resulted in similar muscle lactate concentrations. Following the first 5 to 6 minutes of work, the condition of increased blood pH resulted in a higher arteriovenous lactate difference, a corresponding faster decrease in muscle lactate concentration, and a slower decrease in muscle oxygen uptake and work power per time than the acidotic condition. Their conclusion was that lactate accumulated in the muscle cell and impaired work performance early in the work period, and that the alkaline condition enhanced removal of this limiting metabolite during the ensuing work period, thus decreasing its influence or the influence of H^+ on tension development. This proposed negative

influence of muscle lactate accumulation or decreased pH on muscle tension development is supported by the results of the present study, and also helps to explain the lack of relationship between lactate arteriovenous difference and tension decline at any time during contractions.

The concept of production of large quantities of lactate under conditions of aerobic sufficiency has been proposed (Jobsis and Stainsby, 1968) and opposed (Hirche et al., 1973). Jobsis and Stainsby (1968), utilizing a fluorimetric technique for continuously monitoring the redox state of pyridine nucleotides in mitochondria of canine muscle, illustrated a continued oxidation of NADH during twitch and 5 second tetanic contractions, in spite of lactate production. This result, in conjunction with the measured increased reduction of pyridine nucleotides with forced hypoxia during contractions, supported their claim that lactate was produced in muscle during periods when mitochondria were well-oxygenated. This decrease in reduction of mitochondrial pyridine nucleotides began immediately upon initiation of contractions, thus suggesting the absence of hypoxia even during the initial period of contractile activity. Edington et al. (1973) have more recently shown a progressive increase in muscle lactate concentration to levels as high as 34 μM/g, during 10 minutes of stimulation in rat gastrocnemius muscle, in spite of evidence of well-oxygenated mitochondria. The results of the present study support the concept of lactate production by "aerobic glycolysis" (Hirche et al., 1973), in light of the fact that there was no evidence of mitochondrial hypoxia at 80% of original tension development (2 to 3 minutes from beginning of stimulation) in spite of high muscle lactate levels, negative arteriovenous

lactate differences, and rapid tension decline.

Hirche et al. (1973, 1975) base their claim of no aerobic production of lactate on their observation that estimated lactate production calculated by comparing muscle lactate decrease and lactate arteriovenous difference during the same period, did not occur after the initial period of O_2 deficit. Calculations (Table III) from the results of the present study suggest that, in fact, lactate was being produced in these muscles after the initial hypoxic period during contractions. The time period between the attainment of 50% of original tension and the measurement of muscle lactate at the end of the 50%L experiments was approximately 36 minutes (Table I). The mean lactate decrease in muscle during this time was 0.25 μM/g (Table III). If we assume an average lactate arteriovenous difference during this period of -0.07 μM/g/min (Table III), then the total efflux of lactate from the muscle into the blood during the 36 minute period would approach 2.52 μM/g. Since this estimate of lactate efflux is more than 10 times that which can be accounted for by merely a washing out of muscle accumulated lactate, it must be assumed that lactate is still being produced by the muscle, in spite of evidence of attainment of a metabolic and contractile steady state. The magnitude of lactate production calculated from the present experimental data is probably grossly underestimated, since the lowest lactate arteriovenous difference during this period was assumed to be the average for this period (Table III), and no account was made for disappearance of some of the lactate produced via oxidation by the working muscle, which has been shown to occur using isotopic lactate (Jorfeldt, 1971). It is suggested that the lactate output from the muscle as estimated by Hirche

et al. (1973a) may have involved relatively large errors in estimate due to their failure to account for the rather dramatic changes that appear to occur in plasma water content of the blood perfusing the muscle during high metabolic rates. Following the initial high period of water influx during the first 3 to 10 minutes of contraction (Table III), a subsequent release of this accumulated water from the muscle could produce an apparent underestimate of muscle lactate efflux through a venous dilution effect, which might account for the inconsistency of the results of Hirche et al. with those of the present study.

The attainment of a steady state in muscle ATP concentration after 2 to 3 minutes of contractile activity has been shown to take place relatively independently of work rate (Karlsson et al., 1971). An attainment of steady state ATP concentration by 80% of original tension development occurred in the current investigation. The subsequent ATP level remained statistically unchanged after the 80% interval, in spite of varying degrees of tension decrement at the times when tissue samples were taken (Table VI). This situation, exemplified by the lack of significant correlation between tissue ATP concentration and tension decline, is suggestive of the minor role played by ATP depletion in the tension capabilities of the muscles contracting in this experiment. If it is assumed that the majority of ATP in the muscle cell exists in the cytoplasmic compartment, as is the case in the liver cell (Krebs, 1973), then the muscle tissue appears to maintain a relatively constant level of cytoplasmic ATP, after an original drop to a steady state, which is not limiting to contractile capability. It is understood that metabolite concentration, as

estimated at any given point in time, does not yield direct information concerning metabolic turnover. It can be assumed, nonetheless, that at the level of the contractile machinery, the concentration of ATP immediately before contraction would dictate the tension of the subsequent contraction. If this assumption is valid, a period of rapid tension decrement would be associated with decreased ATP levels, if ATP availability alone were the limiting component. Therefore, the lack of relationship between ATP concentration and tension decrement during contractions supports a concept involving interference of the contractile process, perhaps mediated through lactate accumulation, independent of the availability of ATP. This is further supported by evidence (Cerretelli et al., 1969) that ATP levels during contractile activity can be decreased to levels lower than those measure in the present study.

It is accepted that creatine phosphate (CP), while not constituting a direct energy source for contractile activity, serves as a source of high energy phosphate for the rephosphorylation of ADP (Lohmann, 1934). Decreases in CP as a result of muscular activity are generally more pronounced than decreases in ATP concentration. This phenomenon is most likely due to its facility in transphosphorylating ADP via the creatine kinase reaction (Sahlin et al., 1975), and the necessity of its rephosphorylation only via ATP during the period immeidately following contractile activity (McGilvrey, 1975). As opposed to ATP decrease patterns, CP concentration can approach depletion levels as a result of high intensity contractions under forced hypoxic conditions (Hultman et al., 1967; Karlsson et al., 1971; Fitch et al., 1975). The results of the present study illustrated CP levels above those

reported in muscles contracting at higher metabolic rates (Cerretelli et al., 1969; Edington et al., 1973; Fitch et al., 1975) in spite of resting values which are relatively low compared to data reported from similar preparations (Piiper et al., 1968; Piiper and Spiller, 1970; Hirche et al., 1973). This suggests a sufficiency of high energy phosphate for contractile activity, especially in view of the lack of relationship between rate of tension decline and CP concentration at any point during rhythmic contractions. The fact that CP concentration was significantly related to percent of original tension, and not to rate of tension decline, reflects the stress put on this CP high energy phosphate store during the periods of highest ATP hydrolysis and need for ADP rephosphorylation, during the periods of highest work output.

The cytoplasmic and mitochondrial redox states of the pyridine nucleotides are within the rages outlined by Krebs (1967) in rat liver, illustrating a cytoplasmic $[NAD^+]/[NADH]$ ratio which is much higher than the mitochondrial ratio. During the contraction period, the finding of an unchanged mitochondrial $[NAD^+]/[NADH]$ ratio appears to contradict the results reported by Edington et al. (1973). However, the fact that their muscles showed no apparent fatigue may account for part of this difference, as well as the fact that rat muscles were used in their experiments. Jobsis and Stainsby (1968) have shown increased levels of pyridine nucleotide oxidation during contractions, as have Jobsis and Duffield during very short contractile periods (1967), in both cases utilizing a fluorimetric monitoring technique which is relatively selective for mitochondrial NADH (Jobsis and Duffield, 1967). Edington et al. (1973) claim such an increase in mitochondrial NAD^+

compared to the reduced form is indicative of limitations in the supply of reduced carriers to the respiratory chain, although this limitation was not evident in terms of work performance decrements. The results of the present study suggest an abundant availability of reduced equivalents during contraction, as evidenced by an unchanged mitochondrial $[NAD^+]/[NADH]$. This may be a direct reflection of the low relative work load imposed on the muscle. It would seem that a limitation in the availability of reduced carriers, as reflected by an increase in the estimated $[NAD^+]/[NADH]$ ratio in mitochondria, would exist at work loads approaching maximum aerobic capacity, and not at the submaximal loads used in this study.

The cytoplasmic $[NAD^+]/[NADH]$ showed a significant decrease by 80% of original tension development, which gradually tended to revert towards resting levels as contractions continued. Such a large increase in the reduction state of the cytoplasm is once again inconsistent with the results of Edington et al. (1973), who reported an increased oxidation of the cytoplasmic pyridine nucleotides with contraction. Their increases in $[NAD^+]/[NADH]$ were accompanied by increases in pyruvate which exceed those seen in the present study by, in some cases, a factor of 50. This discrepancy in results of the two studies might be explained by the fact dog gastrocnemius, which is known to have high oxidative potential compared to other species (Maxwell et al., 1975), may have increased capacity in pathways for removing pyruvate, such as pyruvate oxidation by mitochondria and the alanine transaminase reaction (Holloszy et al, 1975). Pyruvate concentrations in the working muscles in the present study did not change significantly from the resting condition, and were similar to those reported from

working human muscle (Karlsson, 1971).

The oxidized form of NAD^+ is a vital component in glycolysis at the step catalyzed by the enzyme glyceraldehyde-3-phosphate dehydrogenase (Newsholme and Start, 1973). The NADH produced via this reaction must be either reoxidized by the coupled conversion of pyruvate to lactate, or via one of the several "shuttles" (Boxer and Devlin, 1961) which are known to function as transport mechanisms of cytoplasm generated electrons into the mitochondria. In attempting to explain the lack of similarity in changes seen in cytoplasmic and mitochondrial $[NAD^+]/[NADH]$, one might be tempted to propose a mechanism whereby the transport of cytoplasmic reduced equivalents into mitochondria via shuttle mechanisms is impaired. However, the results indicated that mitochondrial $[NAD^+]/[NADH]$ remained unchanged, suggesting that, at the workloads imposed in this study, the shuttle of reduced equivalents was sufficient to meet the rate of flux through oxidative phosphorylation.

Increases in ADP concentration in muscle tissue are known to be potent stimulators of glycolysis (Danforth, 1965; Holloszy, 1975). A lag time has been suggested to exist (McGilvrey, 1975) between the production of ADP as a result of contractile activity and its diffusion to the vicinity of the mitochondria. In addition, a translocase has been proposed (Klingenberg, 1970) which includes active transport of ADP into the mitochondrial matrix, in exchange for an ATP molecule. Thus, the massive production of ADP as a product of contractile activity could, in fact, stimulate glycolysis before actually reaching the site of rephosphorylation in the mitochondrial matrix, thus resulting in massive glycolytic flux in the presence of adequate oxygen

supply. Such a concept becomes more feasible when considering that fast twitch fibres use ATP very inefficiently in producing tension (Wendt and Gibbs, 1974), and that these fibres have relatively high glycolytic and low oxidative potential (Peter et al., 1972). It is possible that a major proportion of the tension decrement witnessed in these experiments is a consequence of metabolic occurrences in the working fast twitch fibres, and the accumulation of end-products such as ADP and lactate in these fibres during the working period.

These mechanisms would explain many of the metabolic and contractile phenomena seen in this investigation. At the onset of contraction, the large increases in ADP, as well as Ca^{++} release during stimulation of the muscle, trigger increased glycolytic flux almost immediately (Danforth, 1965; McGilvrey, 1975). Due to inter- and intracell diffusion and transport time of ADP into the mitochondria, ADP increases begin to result in the production of levels of pyruvate not oxidizable by the mitochondria, especially in the contracting fast twitch fibres. Since the mitochondria are under the control of ADP and inorganic phosphate supply, the diffusion and transport time of ADP produces a stimulation of glycolysis and lactate production in fast twitch fibres, while the slow oxidative portion of the muscle is working submaximally. The result is lactate and cytoplasmic NADH accumulation, since NADH cannot be "force fed" into a mitochondrial system under ADP control.

The stimulation of glycolysis by ADP may be amplified out of proportion with concomitant stimulation of aerobic metabolism, as outlined by McGilvrey (1975), a fact which enhances "aerobic lactate production". In addition, it has been proposed that the activity of glycerol-3-phosphate dehydrogenase, which is the enzyme involved in the

shuttle of reduced equivalents in the fast twitch fibre, may be small in this fibre relative to the activities of glycolytic enzymes (McGilvrey, 1975), which would help explain the cytoplasmic accumulation of NADH at the 80% interval.

It is feasible to propose that a limitation in the availability of cytoplasmic NAD^+ at 80% may have been preventing sufficient cytoplasmic ATP production via glycolysis, thus resulting in tension decline. The fact that muscle ATP and CP concentration showed no dramatic alterations after the initial attainment of steady state level fails to support this proposal. In addition, the lack of any glycogen depleted fibres by the 65% sampling interval, in spite of rapid tension decrement to this point, again supports the concept of lactate interference with the contractile process, as opposed to depletion of an energy source vital for contraction.

The metabolic basis for the difference in steady state tensions in the 65%L and 50%L level-off groups is equivocal. The only consistent difference between the groups was a significantly lower muscle lactate concentration in the stimulated versus the control muscle in the 65%L group, which did not appear in the 50%L group data. In addition, the histochemical stains suggested a higher glycogen content in the slow fibres of the 65%L group as opposed to the 50%L group at the end of the contraction period, although no statistical analysis was performed to determine the significance of these differences. This apparent differenct in slow twitch fibre glycogen content may be indicative of the glycogen-sparing effect seen in aerobically trained muscle tissue (Baldwin et al., 1975). Holloszy (1975) proposes that trained muscle tissue can perform aerobic-type work at an intensity

similar to untrained muscle with less glycolysis, due to the fact that the increase in mitochondrial number with training allows less of a buildup in ADP during work, and thus a smaller degree of stimulation of glycolysis. It is conceivable that the muscles of the 65%L group are comparable to aerobically trained muscles in terms of enhanced oxidative capacity, thereby allowing the continuation of contraction at a submaximal metabolic rate with a lesser degree of glycogenolysis. This proposal is supported by the relationship between (A-V) lactate difference at 65% and the level-off tension (Figure 8), and by the significantly lower muscle lactate concentration in the stimulated muscles, as opposed to the resting control muscles, of the 65%L group (Table XIV). Although both the level-off group muscles experienced similar tension decline characteristics during the initial contraction period, the subsequent attainment of steady state tension development was probably related to the degree to which the muscles could phosphorylate ADP in the mitochondria and thus spare fibre glycogen for continued contraction, as the negative influence of the lactate accumulation was being removed. Kugelberg and Edstrom (1968) using stimulated rat muscle, concluded that the initial fast drop in tension seen in their experiments was due to "exhaustion of the glycolytic machinery" of the fast twitch fibres, as suggested by negative PAS reaction in fast twitch fibres at the end of their experiments. This concept is upheld in the present study, with the exception that the initial rapid drop in tension is due to interference with the contractile process in these fibres, while the final steady level tension reached by the contracting muscle is related to the rate and extent of substrate depletion in all fibres.

Proposed Metabolic Mechanisms Associated With Tension Decline

Metabolic evidence in the current investigation suggests that the muscles working under the described experimental conditions were not hypoxic during contractions, as evidenced by the oxygen uptake and blood gas data, and the state of pyridine nucleotides in the mitochondria. In addition, supply for ATP for contraction was apparently constant in spite of altering degrees of tension decline, with additional evidence of adequate phosphorylating power in terms of creatine phosphate concentration throughout the contraction period. Glycogen availability appeared to be adequate during the initial phase of rapid tension decrement, as suggested by the trends seen in the PAS-incubated muscle sections.

The relationships between muscle and arteriovenous lactate parameters and the muscle tension characteristics suggest an influence of lactate accumulation on tension capabilities in working muscle. Decreases in pH are known to affect glycolytic flux adversely (Danforth 1965; Trivedi et al., 1966), especially at the level of phosphofructokinase, as well as the phosphorylase system (Hultman and Bergstrom, 1973). Recent evidence (Sahlin et al., 1975) has also suggested an effect of decreased pH on the creatine kinase reaction, thus influencing the delivery of high-energy phosphate to ADP. Such effects of pH on ATP-supplying mechanisms would be expected to result in a large decrease in both ATP and CP concentration as tension decline was occurring, a phenomenon which was not manifest in the present study. The metabolic evidence lends support to the hypothesis that the lactate accumulation, and perhaps the corresponding decrease in pH, influences muscle contraction by means of a competitive interference, rather than

an effect on enzyme systems responsible for supplying ATP.

Rapid tension decrement occurred during the initial phases of contractile activity in spite of evidence of well-oxygenated mitochondria, as suggested by the oxygen uptake and blood gas data, and by the mitochondrial $[NAD^+]/[NADH]$ ratio estimates. This phenomenon suggests a problem of metabolic communication between the mitochondrion, where ATP is generated aerobically, and the cytoplasm, where ATP is being hydrolyzed during contractions and resynthesized via CP and glycolysis. It is proposed that the major communication problem may occur between the fast and slow twitch fibres, which vary considerably in the rate of ATP utilization during contractions, and in their respective capacities for glycolytic and oxidative metabolism. The contracting fast twitch fibres, when stimulated maximally, may produce ADP in quantities which are within the capabilities of muscle mitochondria to phosphorylate, but which are not immediately available to these mitochondria due to diffusion and transport barriers. The result is stimulation of glycolysis, lactate production, and the subsequent effect of lactate on contractile capabilities. It is worth noting that the level-off tension of these contracting muscles is within the range of per cent composition of slow twitch fibres, suggesting that tension decrement may involve primarily the fast twitch fibre. Although the PAS incubated sections attest to this, experimental relationship between fibre composition and level-off tension was not evident (Appendix E).

It is proposed that the negative effect of muscle-accumulated lactate on fibre contractility decreases gradually as lactate is washed from the muscle, and that the subsequent attainment of a steady state in tension development reflects the muscle's capabilities for

aerobic ADP rephosphorylation. The significant negative relationship between muscle lactate output at 65% of original tension and the level-off tension attained during the longer experiments suggests that the muscles levelling off at the higher tensions were capable of shutting down glycolysis to a greater extent, and at a faster rate, early in the contraction period. Other investigators have examined this "glycogen sparing" characteristic in trained muscle (Baldwin et al., 1975; Holloszy, 1975; Saltin, 1975), and attribute it to the fact that the increased number of mitochondria as a result of training allow the maintenance of relatively low muscle ADP levels during work. The apparent difference (Appendix F) in glycogen content of the fibres of the 65%L and 50%L groups at the end of the contraction period further support this concept.

The massive recruitment of fast twitch fibres during maximum electrical stimulation of muscle is a well known phenomenon (Kugelberg and Edstrom, 1968; Edgerton et al., 1970; Fitts et al., 1973), and was illustrated in the present series of experiments by a rapid glycogen depletion in these fibres, compared to the slow twitch fibres. This pattern of recruitment has been illustrated in vivo as a result of high intensity intermittent isotonic exercise (Gollnick et al., 1973; Armstrong et al., 1974), and during intermittent isometric contractions which are above 20% of maximum voluntary contraction (Gollnick et al., 1974; Hulten et al., 1975). Isotonic exercise of a submaximal prolonged nature, on the other hand, appears to involve fibre recruitment patterns which tend to gradually deplete the glycogen stores of slow twitch fibres (Gollnick et al., 1973; Armstrong et al., 1974). In research involving working muscles which are heterogenous in fibre composition,

one must be aware of the implications of drawing conclusions from muscle tissue concentrations as measured in heterogenous samples. At the present time, the inter-fibre diffusion capabilities of many of the metabolites measured in the present study are unknown, and concentration estimates from tissue plugs may represent mean values representing metabolic extremes occurring in different fibres. Efforts in the future must be made to estimate the metabolic occurrences in individual fibre types in response to contractile activity. In addition, attempts at outlining metabolic factors associated with fatigue in muscle, especially when utilizing the isolated muscle as an experimental model, should aim at simulating as closely as possible the types of contraction that occur in vivo. This will require further understanding of the afferent and efferent neural mechanisms involved in voluntary contractions of various types and intensities.

Summary and Conclusions

Based on the experimental results of the current investigation, the following conclusions appear justified:

1. In maximally contracting muscle, lactate accumulation appears to exert a considerable effect on the ability of the muscle to maintain high tension development during contractions. The resultant rate of decrement in work performance is linearly related to tissue lactate concentration.

2. This effect of lactate accumulation is most likely a direct competitive effect on the contractile machinery, and not merely an index of the degree of hypoxia present. This is illustrated by the fact that ATP, CP and mitochondrial $[NAD^+]/[NADH]$ had reached steady state levels by 80% of original tension, in spite of rapid tension decline

occurring at this interval.

Recommendations

1. It is recommended that future research attempts be aimed at determining the differences in metabolic profiles in the various fibre populations of a muscle performing fatiguing work, and the influence of one fibre type on another in determining total muscle performance.

2. Researchers utilizing isolated muscle preparations should attempt to simulate more closely the types of contractions that occur during voluntary exercise of various types.

3. The systematic investigation of the effects of factors such as "warm-up" and diet manipulation on the contractile and metabolic responses of isolated muscle to rhythmic contractions could assist in extending the limits of human performance during exercise, especially in those types of performance which appear at present to be limited by muscle metabolic profiles.

REFERENCES

Ahlborg, B., Bergstrom, J., Ekelund, L., Guarnieri, G., Harris, R., Hultman, E., and Nordesjo, L. Muscle metabolism during isometric exercise performed at constant force. J. Appl. Physiol. 33: 224-228, 1972.

Ahlborg, B., Bergstrom, J., Ekelund, L., and Hultman, E. Muscle glycogen and muscle electrolytes during prolonged physical exercise. Acta Physiol. Scand. 70: 129-142, 1967.

Ariano, M., Armstrong, R., and Edgerton, V. Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21: 51-55, 1973.

Arinze, I. and Hanson, R. Mitochondrial redox state and the regulation of gluconeogenesis in the isolated, perfused cat liver. F.E.B.S. Letters 31: 280-282, 1973.

Armstrong, R., Saubert, C., Sembrowich, W., Shepherd, R., and Gollnick, P. Glycogen depletion in rat skeletal muscle fibers during exercise. Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. Poortmans. Switzerland: Birkhauser Verlag Basel, 1975.

Armstrong, R., Saubert, C., Sembrowich, W., Shepherd, R., and Gollnick, P. Glycogen depletion in rat skeletal muscle fibers at different intensities and durations of exercise. Pflugers Arch. 352: 243-256, 1974.

Astrand, P., Hallback, I., Hedman, R., and Saltin, B. Blood lactates after prolonged severe exercise. J. Appl. Physiol. 18: 619-622, 1963.

Baldwin, K., Fitts, R., Booth, F., Winder, W., and Holloszy, J. Depletion of muscle and liver glycogen during exercise. Protective effect of training. Pflugers Arch. 354: 203-212, 1975.

Baldwin, K., Reitman, J., Terjung, R., Winder, W., and Holloszy, J. Substrate-depletion in different types of muscle and in liver during prolonged running. Am. J. Physiol. 225: 1045-1050, 1973.

Baldwin, K. and Tipton, C. Work and metabolic patterns of fast and slow twitch skeletal muscle contracting in situ. Pflugers Arch. 334: 345-356, 1972.

Barclay, J., Allen, P., and Stainsby, W. The relationship between temperature and oxygen uptake of contracting skeletal muscle. Med. Sci. Sports 6: 33-37, 1974.

Barclay, J. and Stainsby, W. Intramuscular lipid store utilization by contracting dog skeletal muscle in situ. Am. J. Physiol.

223: 115-119, 1972.

Barclay, J. and Stainsby, W. The role of blood flow in limiting maximal metabolic rate in muscle. Med. Sci. Sports 7: 116-119, 1975.

Bergmeyer, H. Methods of Enzymatic Analysis. New York: Academic Press, 1965.

Bergstrom, J., Guarnieri, G., and Hultman, E. Carbohydrate metabolism and electrolyte changes in human muscle tissue during heavy work. J. Appl. Physiol. 30: 122-125, 1971.

Bergstrom, J., Guarnieri, G., and Hultman, E. Changes in muscle water and electrolytes during exercise. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Bergstrom, J., Harris, R., Hultman, E., and Nordesjo, L. Energy-rich phosphagens in dynamic and static work. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Bergstrom, J., Hermansen, L., Hultman, E., and Saltin, B. Diet, muscle glycogen and physical performance. Acta Physiol. Scand. 71: 140-150, 1967.

Borg, G. Perceived exertion: a note on history and methods. Med. Sci. Sports 5: 90-93, 1973.

Bowers, W., Hubbard, R., Smoake, J., Daum, R., and Nilson, E. Effects of exercise on the ultra-structure of skeletal muscle. Am. J. Physiol. 227: 313-316, 1974.

Boxer, G. and Devlin, T. Pathways of intracellular hydrogen transport. Science 10: 1495-1500, 1961.

Brooks, G., Hittelman, K., Faulkner, J., and Beyer, R. Temperature, skeletal muscle mitochondrial functions, and oxygen debt. Am. J. Physiol. 220: 1053-1059, 1971.

Burke, R., Levine, D., Zajac, F., Tsairis, P., and Engel, W. Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. Science 174: 709-712, 1971.

Carlson, L., Ekelund, L., and Froberg, S. Concentration of triglycerides, phospholipids and glycogen in skeletal muscle and of free fatty acids and -hydroxybutyric acid in blood in man in response to exercise. Europ. J. Clin. Invest. 1: 248-254, 1971.

Cerretelli, P., DiPrampero, P., and Piiper, J. Energy balance of anaerobic work in the dog gastrocnemius muscle. Am. J. Physiol. 217: 581-585, 1969.

Chagovets, N. Adenine nucleotides, NAD^+, and NADH in skeletal muscles during intensive work and in a period of rest. Akademiia Nauk SSSR Doklady: Biochemistry 207: 493-495, 1972.

Chapler, C. and Katrusiak, J. Blood flow and carbohydrate metabolism in dog skeletal muscle in situ following propranolol. Med. Sci. Sports 6: 193-197, 1974.

Chapler, C. and Moore, W. Glycogen distribution in dog skeletal muscle. J. Appl. Physiol. 32: 542-545, 1972.

Chapler, C. and Stainsby, W. Carbohydrate metabolism in contracting dog skeletal muscle in situ. Am. J. Physiol. 215: 995-1004, 1968.

Corsi, A., Midrio, M., and Granata, A. In situ utilization of glycogen and blood glucose in skeletal muscle during tetanus. Am. J. Physiol. 216: 1534-1541, 1969.

Costill, D., Bowers, R., Branam, G., and Sparks, K. Muscle glycogen utilization during prolonged exercise on successive days. J. Appl. Physiol. 31: 834-838, 1971.

Costill, D., Gollnick, P., Jansson, E., Saltin, B., and Stein, E. Glycogen depletion pattern in human muscle fibers during distance running. Acta Physiol. Scand. 89: 374-383, 1973.

Costill, D. and Saltin, B. Muscle glycogen and electrolytes following exercise and thermal dehydration. Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. Poortmans. Switzerland: Birkhauser Verlag Basel, 1975.

Costill, D., Sparks, K., Gregor, R., and Turner, C. Muscle glycogen utilization during exhaustive running. J. Appl. Physiol. 31: 353-356, 1971.

Costill, D., Thomason, H., and Roberts, E. Fractional utilization of the aerobic capacity during distance running. Med. Sci. Sports 5: 248-252, 1973.

Costin, J., Saltin, B., Skinner, N. and Vastagh, G. Glucose uptake at rest and during contraction in isolated dog skeletal muscle. Acta Physiol. Scand. 81: 124-137, 1971.

Danforth, W. Activation of glycolytic pathway in muscle. Control of Energy Metabolism. Edited by B. Chance et al. New York: Academic Press, 1965.

Danforth, W. and Helmreich, E. Regulation of glycolysis in muscle. I. The conversion of phosphorylase b to phosphorylase a in frog sartorius muscle. J. Biol. Chem. 239: 3133-3138, 1964.

Davidsohn, I. and Henry, J. Clinical Diagnosis. Philadelphia: W.B. Saunders, 1969.

Dawson, C., Nadel, E. and Horvath, S. Arterial blood and muscle lactates during swimming in the rat. J. Appl. Physiol. 30: 322-327, 1971.

DiPrampero, P., Cerretelli, P., and Piiper, J. Lactic acid formation in gastrocnemius muscle of the dog and its relation to oxygen debt contraction. Resp. Physiol. 8: 347-353, 1969.

DiPrampero, P., Cerretelli, P., and Piiper, J. O_2 consumption and metabolite balance in the dog gastrocnemius at rest and during exercise. Pflugers Arch. 309: 38-47, 1969.

Dohm, G., Huston, R., and Askew, E. Effects of hypoxia on oxidative capacity of skeletal muscle in trained and untrained rats. Proc. Soc. Exptl. Biol. Med. 142: 977-980, 1973.

Dunn, R. and Critz, J. Effect of circulating FFA on lactate production by skeletal muscle during stimulation. J. Appl. Physiol. 38: 801-805, 1975.

Duran, W. and Renkin, E. Oxygen consumption and blood flow in resting mammalian skeletal muscle. Am. J. Physiol. 226: 173-177, 1974.

Eberstein, A. and Sandow, A. Fatigue mechanisms in muscle fibers. The Effect of Use and Disuse on Neuromuscular Functions. Edited by E. Gutmann and P. Hnik. Amsterdam: Elsevier Publishing Co., 1963.

Edgerton, V., Barnard, R., Peter, R., Simpson, D., and Gillespie, C. Response of muscle glycogen and phosphorylase to electrical stimulation in trained and nontrained guinea pigs. Exptl. Neurol. 27: 46-56, 1970.

Edington, D. Pyridine nucleotide oxidized to reduced ratio as a regulator of muscle performance. Experientia 26: 601-602, 1970.

Edington, D., Ward, G., and Saville, W. Energy metabolism of working muscle: concentration profiles of selected metabolites. Am. J. Physiol. 224: 1375-1380, 1973.

Edwards, R., Harris, R., Hultman, E., Kaijser, L., Koh, D., and Nordesjo, L. Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions sustained to fatigue, of the quadriceps muscle in man. J. Physiol. 220: 335-352, 1972.

Fagraeus, L., Karlsson, J., Linnarsson, D., and Saltin, B. Oxygen uptake during maximal work at lowered and raised ambient air pressures. Acta Physiol. Scand. 87: 411-421, 1973.

Feigl, E. and D'Alecy, L. Normal arterial blood pH, oxygen, and carbon dioxide tensions in unanesthetized dogs. J. Appl. Physiol. 32: 152-153, 1972.

Fitch, C., Jellinek, M., Fitts, R., Baldwin, K., and Holloszy, J. Phosphorylated -quanidino-propionate as a substitute for phosphocreatine in rat muscle. Am. J. Physiol. 228: 1123-1125, 1975.

Fitts, R., Booth, F., Winder, W., and Holloszy, J. Skeletal muscle respiratory capacity, endurance, and glycogen utilization. Am. J. Physiol. 228: 1029-1033, 1975.

Fitts, R., Campion, D., Nagle, F., and Cassens, R. Contractile properties of skeletal muscle from trained miniature pig. Pflugers Arch. 343: 133-141, 1973.

Folbergrova, J., MacMillan, B., and Siesjo, B. The effect of moderate and marked hypercapnia upon the energy state and upon the cytoplasmic NADH/NAD^+ ratio of the rat brain. J. Neurochem. 19: 2497-2505, 1972.

Froberg, S. Effect of acute exercise on tissue lipids in rats. Metabolism 20: 714-720, 1971.

Froberg, S., Carlson, L., and Ekelund, L. Local lipid stores and exercise. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Froberg, S. and Mossfeldt, F. Effect of prolonged strenuous exercise on the concentration of triglycerides, phospholipids and glycogen in muscle of man. Acta Physiol. Scand. 82: 167-171, 1971.

Fuchs, F., Reddy, Y., and Briggs, F. The interaction of cations with the calcium-binding site of troponin. Biochim. Biophys. Acta 221: 407-409, 1970.

Gale, J. Mitochondrial swelling associated with exercise and method of fixation. Med. Sci. Sports 6: 182-187, 1974.

Gergely, J. The regulatory proteins of myofibrils and their role in the mechanism of contraction and relaxation. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Goldstein, L. and Harley-DeWitt, S. Renal gluconeogenesis and mitochondrial NAD^+/NADH ratios in nursing and adult rats. Am. J. Physiol. 224: 752-757, 1973.

Gollnick, P., Armstrong, R., Saltin, B., Saubert, C., Sembrowich, W., and Shepherd, R. Effect of training on enzyme activity and fiber composition of human skeletal muscle. J. Appl. Physiol. 34: 107-111, 1973.

Gollnick, P., Armstrong, R., Saubert, G., Piehl, K., and Saltin, B. Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J. Appl. Physiol. 33: 312-319, 1972.

Gollnick, P., Armstrong, R., Saubert, C., Sembrowich, W., Shepherd, R., and Saltin, B. Glycogen depletion patterns in human skeletal muscle fibers during prolonged work. Pflugers Arch. 344: 1-12, 1973.

Gollnick, P., Armstrong, R., Sembrowich, W., Shepherd, R., and Saltin, B. Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise. J. Appl. Physiol. 34: 615-618, 1973.

Gollnick, P., Ianuzzo, C. and King, D. Ultrastructural and enzyme changes in muscles with exercise. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Gollnick, P., Karlsson, J., Piehl, K., and Saltin, B. Selective glycogen depletion in skeletal muscle fibres of man following sustained contractions. J. Physiol. 241: 59-67, 1974.

Gollnick, P. and King, D. Effect of exercise and training on mitochondria of rat skeletal muscle. Am. J. Physiol. 216: 1502-1509, 1969.

Gollnick, P., Piehl, K., Saubert, C., Armstrong, R., and Saltin, B. Diet, exercise, and glycogen changes in human muscle fibers. J. Appl. Physiol. 33: 421-425, 1972.

Gollnick, P., Struck, P., and Bogyo, T. Lactic dehydrogenase activities of rat heart and skeletal muscle after exercise and training. J. Appl. Physiol. 22: 623-627, 1967.

Gonzalez-Serratos, H., Borrero, L., and Franzini-Armstrong, C. Changes in mitochondria and intracellular pH during muscle fatigue. Fed. Proc. 33: 1401, 1974.

Granit, R. and Burke, R. The control of movement and posture. Brain Research 53: 1-28, 1973.

Gubjarnason, S., Mathes, P. and Ravens, K. Functional compartmentation of ATP and creatine phosphate in heart muscle. J. Mol. Cell. Cardiol. 1: 325-339, 1970.

Hagenfeldt, L. and Wahren, J. Human forearm muscle metabolism during exercise. III. Uptake, release and oxidation of β-hydroxybutyrate and observations on the β-hydroxybutyrate/acetoacetate ratio. Scand. J. Clin. Lab. Invest. 21: 314-320, 1968.

Hagenfeldt, L. and Wahren, J. Metabolism of free fatty acids and ketone bodies in skeletal muscle. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Hannerz, J. Discharge properties of motor units in man. Experientia 29: 45-46, 1973.

Hansford, R. The control of tricarboxylate-cycle oxidations in blowfly flight muscle. Biochem J. 146: 537-547, 1975.

Harper, H. Review of Physiological Chemistry. Los Altos: Lange Medical Publications, 1971.

Hemms, D. and Gaja, G. Carbohydrate metabolism in the isolated perfused rat kidney. Biochem. J. 128: 421-426, 1972.

Henneman, E. and Olson, C. Relations between structure and function in the design of skeletal muscle. J. Neurophysiol. 28: 581-598, 1965.

Henry, R., Cannon, D., and Winkelman, J. Clinical Chemistry: Principles and Technics. Hagerstown, Md.: Harper and Tow, 1974.

Hermansen, L., Hultman, E., and Saltin, B. Muscle glycogen during prolonged severe exercise. Acta Physiol. Scand. 71: 129-139, 1967.

Hermansen, L. and Osnes, J. Blood and muscle pH after maximal exercise in man. J. Appl. Physiol. 32: 304-308, 1972.

Hirche, H., Bovenkamp, U., Busse, J., Hombach, B., and Manthey, J. Factors influencing lactic acid production in working skeletal muscle. Scand. J. Clin. Lab. Invest. Suppl. 128, Vol. 31: 11-15, 1973.

Hirche, H., Grun, D., and Waller, W. Utilization of carbohydrates and free fatty acids by the gastrocnemius of the dog during long lasting rhythmical exercise. Pflugers Arch. 321: 121-132, 1970.

Hirche, H., Hombach, V., Langohr, H., Wacker, U., and Busse, J. Lactic acid permeation rate in working gastrocnemii of dogs during metabolic alkalosis and acidosis. Pflugers Arch. 356: 209-222, 1975.

Hirche, H., Langohr, J., Oswald, S., and Wacker, U. A new technique for rapid freezing of tissue specimens from isolated skeletal muscles. Pflugers Arch. 343: 89-94, 1973.

Hirche, H., Langohr, H. and Wacker, U. Lactic acid accumulation in working skeletal muscle. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Hirche, H., Raff, W., and Grun, D. The resistance to blood flow in the gastrocnemius of the dog during sustained and rhythmical isometric and isotonic contractions. Pflugers Arch. 314: 97-112, 1970.

Holloszy, J. Adaptation of skeletal muscle to endurance exercise. Med. Sci. Sports 7: 155-164, 1975.

Holloszy, J., Booth, F., Winder, W., and Fitts, R. Biochemical adaptation of skeletal muscle to prolonged physical exercise. Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. Poortmans. Switzerland: Birkhauser Verlag Basel, 1975.

Holloszy, J., Mole, P., Baldwin, K., and Terjung, R. Exercise induced enzymatic adaptations in muscle. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Hoppeler, H., Luthi, P., Claassen, H., Weikel, E., and Howald, H. The ultrastructure of normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. Pflugers Arch. 344: 217-232, 1973.

Hubbard, J. The effect of exercise on lactate metabolism. J. Physiol. 231: 1-18, 1973.

Hudlicka, O. Differences in development of fatigue in slow and fast muscles. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Hulten, B., Thortensson, A., Sjodin, B., and Karlsson, J. Relationship between isometric endurance and fibre types in human leg muscles. Acta Physiol. Scand. 93: 135-138, 1975.

Hultman, E. and Bergstrom, J. Local energy-supplying substrates as limiting factors in different types of leg muscle work in normal man. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Hultman, E., Bergstrom, J., and Anderson, N. Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with muscular work in man. Scand. J. Clin. Lab. Invest. 19: 56-66, 1967.

Hultman, E., Bergstrom, J., and Roch-Norlund, A. Glycogen storage in human skeletal muscle. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Issekutz, B. and Paul, P. Intramuscular energy sources in exercising normal and pancreatectomized dogs. Am. J. Physiol. 215: 197-204, 1968.

Jacobsson, S. and Kjellmer, I. Accumulation of fluid in exercising skeletal muscle. Acta Physiol. Scand. 60: 286-292, 1964.

Jobsis, F. and Duffield, J. Oxidative and glycolytic recovery metabolism in muscle. Fluorometric observations on their relative contributions. J. Gen. Physiol. 50: 1009-1047, 1967.

Jobsis, F. and Stainsby, W. Oxidation of NADH during contractions of circulated mammalian skeletal muscle. Resp. Physiol. 4: 292-300, 1968.

Johnson, R. and Hansford, R. The control of tricarboxylate-cycle oxidations in blowfly flight muscle. The steady-state concentrations of citrate, isocitrate, 2-oxoglutarate and malate in flight muscle and isolated mitochondria. Biochem. J. 146: 52 527-535, 1975.

Karlsson, J. Lactate and phosphagen concentrations in working muscle of man. Acta Physiol. Scand. Suppl. 358, 1971.

Karlsson, J. Pyruvate and lactate ratios in muscle tissue and blood during exercise in man. Acta Physiol. Scand. 81: 455-458, 1971.

Karlsson, J., Diamant, B. and Saltin, B. Lactate dehydrogenase activity in muscle after prolonged severe exercise in man. J. Appl. Physiol. 25: 88-91, 1968.

Karlsson, J., Diamant, B., and Saltin, B. Muscle metabolites during submaximal and maximal exercise in man. Scand. J. Clin. Lab. Invest. 26: 385-394, 1971.

Karlsson, J., Funderburk, C., Essen, B., and Lind, A. Constituents of human muscle in isometric fatigue. J. Appl. Physiol. 38: 208-211, 1975.

Karlsson, J., Frith, K., Sjodin, B., Gollnick, P., and Saltin, B. Distribution of LDH isozymes in human skeletal muscle. Scand. J. Clin. Lab. Invest. 33: 307-312, 1974.

Karlsson, J., Nordesjo, L., Jorfeldt, L., and Saltin, B. Muscle lactate, ATP and CP levels during exercise after physical training in man. J. Appl. Physiol. 33: 199-203, 1972.

Karlsson, J. and Ollander, B. Muscle metabolites and exhaustive static exercise of different duration. Acta Physiol. Scand. 86: 309-314, 1972.

Karlsson, J. and Saltin, B. Lactate, ATP, and CP in working muscles during exhaustive exercise in man. J. Appl. Physiol. 29: 598-602, 1970.

Karlsson, J. and Saltin, B. Oxygen deficit and muscle metabolites in intermittent exercise. Acta Physiol. Scand. 82: 115-122, 1971.

Katz, A. Contractile proteins of the heart. Physiol. Rev. 50: 63-158, 1970.

Keul, J., Doll, E., and Keppler, D. The substrate supply of the human skeletal muscle at rest, during and after work. Experientia 23: 975-979, 1964.

Keul, J., Doll, E.,and Keppler, D. Energy Metabolism of Human Muscle. Baltimore: University Park Press, 1972.

Keul, J., Haralambie, G. and Trittin, G. Intermittent exercise: arterial lipid substrate and arteriovenous differences. J. Appl. Physiol. 36: 159-162, 1974.

Khanna, J., Kalant, H., and Roth, J. Effect of chronic intake of ethanol on lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios in rat liver. Can. J. Physiol. Pharmacol. 53: 299-303, 1975.

Klingenberg, M. Metabolite transport in mitochondria: an example for intracellular membrane function. Essays in Biochemistry 6: 119-160, 1970.

Kraupp, O., Alder-Kastner, L., Niessner, H., and Plank, B. The effects of starvation and of acute and chronic alloxan diabetes on myocardial substrate levels and on liver glycogen in the rate in vivo. Eur. J. Biochem. 2: 197-214, 1967.

Krebs, H. Pyridine nucleotides and rate control. Rate Control in Biological Processes. Cambridge: University Press, 1973.

Krebs, H. Role of the redox state of nicotinamide adenine dinucleotides in the regulation of metabolic processes. National Cancer Institute Monograph 27: 331-343, 1967.

Krebs, H., Mellanby, J., and Williamson, D. The equilibrium constant of the β-hydroxybutyric dehydrogenase system. Biochem. J. 82: 96-98, 1962.

Kugelberg, E. and Edstrom, L. Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres: relation to fatigue. J. Neurol. Neurosurg. Psychiat. 31: 415-423, 1968.

Lehninger, A. Biochemistry. New York: Worth Publishers, 1972.

Lehninger, A., Sudduth, H., and Wise, J. D-β-hydroxybutyric dehydrogenase of mitochondria. J. Biol. Chem. 235: 2450-2455, 1960.

Lindholm, A., Bjerneld, H., and Saltin, B. Glycogen depletion pattern in muscle fibers of trotting horses. Acta Physiol. Scand. 90: 475-484, 1974.

Linnarsson, D., Karlsson, J., Fagraeus, L., and Saltin, B. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. J. Appl. Physiol. 36: 399-402, 1974.

Lo, S., Russell, J., and Taylor, A. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28: 234-236, 1970.

Lohmann, K. Uber die enzymatische Alfspaltung der Kreatinphosphorsaure; zugleich ein Beitrag zum Chemismus der Muskelkontraktion. Biochem. Z. 271: 264, 1934.

Lowenstein, J. Citric Acid Cycle Control and Compartmentation. New York: Marcel Dekker, 1969.

Lowry, O. and Passonneau, J. A Flexible System of Enzymatic Analysis New York: Academic Press, 1972.

Lundquist, C. and Kiessling, K. Oxidation of DL-α-glycerophosphate and β-hydroxybutyrate in red and white skeletal muscle. Acta Physiol. Scand. 21: 1385-1386, 1967.

Mainwood, G., Worsley-Brown, P., and Paterson, R. The metabolic changes in frog sartorius muscles during recovery from fatigue at different external bicarbonate concentrations. Can. J. Physiol. Pharmacol. 50: 143-155, 1971.

Masoro, E., Rowell, L., McDonald, R., and Steiert, B. Skeletal muscle lipids. II. Nonutilization of intracellular lipid esters as an energy source for contractile activity. J. Biol. Chem. 241: 2626-2634, 1966.

Maxwell, L., Faulkner, J., Mohrman, D., and Barclay, J. Physiology of dog and cat skeletal muscle. Twenty-second Annual Meeting, A.C.S.M. New Orleans, 1975.

McGilvery, R. The use of fuels for muscular work. Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. Poortmans, Switzerland: Birkhauser Verlag Basel, 1975.

Merton, P. Voluntary strength and fatigue. J. Physiol. 123: 553-564, 1954.

Miller, E., Ben, M., and Cass, J. Comparative anesthesia in laboratory animals. Fed. Proc. 28: 1373-1586, 1969.

Miller, M., Christensen, G., and Evans, H. Anatomy of the Dog. Philadelphia: W. B. Saunders Co., 1964.

Milner-Brown, H., Stein, R. and Yemm, R. Changes in firing rate of human motor units during linearly changing voluntary contractions. J. Physiol. 230: 371-390, 1973.

Milner-Brown, H., Stein, R. and Yemm, R. The orderly recruitment of human motor units during voluntary isometric contractions. J. Physiol. 230: 359-370, 1973.

Mintz, S. and Robin, E. Redox state of free nicotinamide adenine nucelotides in the cytoplasm and mitochondria of alveolar macrophages. J. Clin. Invest. 50: 1181-1186, 1971.

Mitchelson, K. and Hird, F. Effect of pH and halothane on muscle and liver mitochondria. Am. J. Physiol. 225: 1393-1398, 1973.

Morganroth, M., Mohrman, D., and Sparks, H. Prolonged vasodilation following fatiguing exercise of dog skeletal muscle. Am. J. Physiol. 229: 38-43, 1975.

Neptune, E., Sudduth, H., Fash, F., and Reish, J. Metabolism of β-hydroxybutyrate and acetoacetate by excised rate diaphragm and diaphragm homogenate. Am. J. Physiol. 201: 235-238, 1961.

Newsholme, E. and Start, C. Regulation in Metabolism. New York: John Wiley and Sons, 1973.

Otto, D. and Ontko, J. Regulation of the mitochondrial oxidation-reduction state in intact hepatocytes by calcium ions. Biochem. Biophys. Res. Comm. 61: 743-750, 1974.

Parrika, R. and Goodman, M. Nitrogen metabolism in the isolated perfused rat liver. Biochem. J. 138: 341-348, 1974.

Pearse, A. Histochemistry, Theoretical and Applied. London: J.A. Churchill, Ltd., 1969.

Peter, J. Histochemical, biochemical, and physiological studies of skeletal muscle and its adaptation to exercise. Contractility of Muscle Cells and Related Processes. Edited by R. Podolsky. Englewood Cliffs, N.J.: Prentice-Hall, 1971.

Peter, J., Barnard, R., Edgerton, V., Gillespie, C., and Stempel, K. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochemistry 11: 2627-2633, 1972.

Piehl, K. Glycogen storage and depletion in human skeletal muscle fibers. Acta Physiol. Scand. Suppl. 402, 1974.

Piehl, K. Time course for refilling of glycogen stores in human muscle fibres following exercise-induced glycogen depletion. Acta Physiol. Scand. 90: 297-302, 1974.

Piiper, J., DiPrampero, P., and Cerretelli, P. Oxygen debt and high-energy phosphates in gastrocnemius muscle of the dog. Am. J. Physiol. 215: 523-531, 1968.

Piiper, J., DiPrampero, P. and Cerretelli, P. Oxygen consumption and mechanical performance of dog gastrocnemius muscle with artificially increased blood flow. Pflugers Arch. 331: 312-325, 1969.

Piiper, J. and Spiller, P. Repayment of O_2 debt and resynthesis of high-energy phosphates in gastrocnemius muscle of the dog. J. Appl. Physiol. 28: 657-662, 1970.

Pinelli, A. A new colorimetric method for plasma fatty acid analysis. Clin. Chim. Acta 44: 385-390, 1973.

Poulus, A., Docter, H., and Westra, H. Acid-base balance and subjective feelings of fatigue during physical exercise. Europ. J. Appl. Physiol. 33: 207-213, 1974.

Purvis, J. and Lowenstein, J. The relation between intra- and extra-mitochondrial pyridine nucleotides. J. Biol. Chem. 236: 2794-2803, 1961.

Reitman, J., Baldwin, K. and Holloszy, J. Intramuscular triglyceride utilization of red, white, and intermediate skeletal muscle and heart during exhausting exercise. Proc. Soc. Exptl. Biol. Med. 142: 628-631, 1973.

Sacktor, B., Wormser-Shavit, E., and White, J. Diphosphopyridine nucleotide-linked cytoplasmic metabolites in rat leg muscle in situ during contraction and recovery. J. Biol. Chem. 240: 2678-2681, 1965.

Sahlin, K., Harris, R., and Hultman, E. Creatine kinase equilibirum and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. Biochem J. 152: 173-180, 1975.

Saiki, H., Margaria, R., and Cuttica, F. Lactic acid production in submaximal work. Int. Z. angew. Physiol. 24: 57-61, 1967.

Saltin, B. Metabolic fundamentals in exercise. Med. Sci. Sports 5: 137-146, 1973.

Saltin, B. Adaptive changes in carbohydrate metabolism with exercise. Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. Poortmans. Switzerland: Birkhauser Verlag Basel, 1975.

Saltin, B., Gagge, A., Bergh, U., and Stolwijk, J. Body temperatures and sweating during exhaustive exercise. J. Appl. Physiol. 32: 635-643, 1972.

Saltin, B. and Karlsson, J. Muscle glycogen utilization during work at different intensities. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Schafer, G. Efficiency and capacity of mitochondrial energy transformation. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Schlein, E., Jensen, D., and Knochel, J. Effect of plasma water loss on assessment of muscle metabolism during exercise. J. Appl. Physiol. 34: 568-572, 1973.

Sigma Chemical Company. Pyruvic acid and lactic acid at 340 mμ. Technical Bulletin No. 726-UV and No. 826-UV. Sigma Chemical Company, St. Louis, Mo., 1968.

Sigma Chemical Company. The colorimetric determination of glucose in whole blood, plasma or serum at 620-650 nm. Technical Bulletin No. 635. Sigma Chemical Company, St. Louis, Mo., 1973.

Simonson, E. Physiology of Work Capacity and Fatigue. Springfield: Charles C. Thomas, 1971.

Skinner, N., Costin, J., Saltin, B., and Vastagh, G. Glucose uptake in contracting, isolated in situ dog skeletal muscle. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Spande, J. and Schottelius, B. Chemical basis of fatigue in isolated mouse soleus muscle. Am. J. Physiol. 219: 1490-1495, 1970.

Stainsby, W. Critical oxygen tensions in muscle. Limiting Factors of Physical Performance. Edited by J. Keul. Stuttgart: Georg Thieme, 1973.

Stainsby, W. Oxygen uptake for isotonic and isometric twitch contractions of dog skeletal muscle in situ. Am. J. Physiol. 219: 435-439, 1970.

Stainsby, W. and Barclay, J. Oxygen uptake for brief tetanic contractions of dog skeletal muscle in situ. Am. J. Physiol. 223: 371-375, 1972.

Stainsby, W. and Barclay, J. Relation of load, rest length, work, and shortening to oxygen uptake by in situ dog semitendinosus. Am. J. Physiol. 221: 1238-1242, 1971.

Stainsby, W. and Fales, J. Oxygen consumption for isometric tetanic contractions of dog skeletal muscle in situ. Am. J. Physiol. 224: 687-691, 1973.

Stainsby, W. and Otis, A. Blood flow, blood oxygen tension, oxygen uptake, and oxygen transport in skeletal muscle. Am. J. Physiol. 206: 858-866, 1964.

Stainsby, W. and Welch, H. Lactate metabolism of contracting dog skeletal muscle in situ. Am. J. Physiol. 211: 177-183, 1966.

Stambaugh, R. and Post, D. Substrate and product inhibition of rabbit muscle lactic dehydrogenase heart (H_4) and muscle (M_4) isozymes. J. Biol. Chem. 241: 1462-1467, 1966.

Stowe, D., Owen, T., Anderson, D., Haddy, F., and Scott, J. Interaction of O_2 and CO_2 in sustained exercise hyperemia of canine skeletal muscle. Am. J. Physiol. 229: 28-33, 1975.

Stumpfhauser, L. and Lamb, D. Glycogen content and glucose uptake in stimulated frog sartorius muscle: effects of prolonged incubation insulin and testosterone. Int. Z. angew. Physiol. 31: 163-172, 1972.

Sugrobova, N., Kurganov, B., and Yakovlev, V. Role of lactate dehydrogenase system in stabilization of ratio of oxidized to reduced form of NAD. Biorak 39: 516-519, 1974.

Sund, H. Pyridine Nucleotide-Dependent Dehydrogenases. New York: Heidelberg, 1970.

Taylor, A., Lappage, R. and Rao, S. Skeletal muscle glycogen stores after submaximal and maximal work. Med. Sci. Sports 3: 75-78, 1971.

Taylor, A. and Rao, S. Rat blood and muscle lactate after prolonged exercise. Can. J. Physiol. Pharmacol. 51: 277-283, 1973.

Taylor, A., Thayer, R. and Rao, S. Human skeletal muscle glycogen synthetase activities with exercise and training. Can. J. Physiol. Pharmacol. 50: 411-415, 1972.

Terjung, R., Baldwin, K., Mole, P., Klinkerfuss, G., and Holloszy, J. Effect of running to exhaustion on skeletal muscle mitochondria: a biochemical study. Am. J. Physiol. 223: 549-554, 1972.

Tobin, R., Mackerer, C., and Mehlman, M. pH effects on oxidative phosphorylation of rat liver mitochondria. Am. J. Physiol. 223: 83-88, 1972.

Trivedi, B. and Danforth, W. Effect of pH on the kinetics of frog muscle phosphofructokinase. J. Biol. Chem. 241: 4110-4112, 1966.

Vesell, E. and Pool, P. Lactate and pyruvate concentrations in exercised ischemic canine muscle: relationship of tissue substrate level to lactate dehydrogenase isozyme pattern. Proc. Nat. Acad. Sci. 55: 756-762, 1966.

Wahren, J., Ahlborg, G., Felig, P., and Jorfeldt, L. Glucose metabolism during exercise in man. Muscle Metabolism During Exercise. Edited by B. Pernow and B. Saltin. New York: Plenum Press, 1971.

Wahren, J., Felig, P., Hendler, R., and Ahlborg, G. Glucose and amino acid metabolism during recovery after exercise. J. Appl. Physiol. 34: 838-845, 1973.

Welch, H., Mullin, J., Wilson, G., and Lewis, J. Effects of breathing O_2-enriched gas mixtures on metabolic rate during exercise. Med. Sci. Sports 6: 26-32, 1974.

Welch, H. and Stainsby, W. Oxygen debt in contracting dog skeletal muscle in situ. Resp. Physiol. 3: 229-242, 1967.

Wendt, I. and Gibbs, C. Energy production of mammalian fast- and slow-twitch muscles during development. Am. J. Physiol. 226: 642-647, 1974.

Whalen, W., Buerk, D., and Thuning, C. Blood flow-limited oxygen consumption in resting cat skeletal muscle. Am. J. Physiol. 224: 763-768, 1973.

Williamson, D., Bates, M., Page, M., and Krebs, H. Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem. J. 121: 41-47, 1971.

Williamson, D. and Hems, R. Metabolism and function of ketone bodies. Essays in Cell Metabolism. Edited by W. Bartley, H. Kornberg and J. Quayle. London: Wiley Interscience, 1970.

Williamson, D., Lund, P., and Krebs, H. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem. J. 103: 514-527, 1969.

Williamson, D., Mellanby, J., and Krebs, H. Enzymic determination of D-(-)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem. J. 82: 90-96, 1962.

Wilson, B., Welch, H. and Liles, J. Effects of hyperoxic gas mixtures on energy metabolism during prolonged work. J. Appl. Physiol. 39: 267-271, 1975.

Wilson, D., Stubbs, M., Oshino, N., and Erecinska, M. Thermodynamic relationships between the mitochondrial oxidation-reduction reactions and cellular ATP levels in ascites tumor cells and perfused rat liver. Biochemistry 13: 5305-5311, 1974.

Wilson, G. and Welch, H. Effects of hyperoxic gas mixtures on exercise tolerance in man. Med. Sci. Sports 7: 48-52, 1972.

Winder, W., Baldwin, K., and Holloszy, J. Enzymes involved in ketone utilization in different types of muscle: adaptation to exercise. Eur. J. Biochem. 47: 461-467, 1974.

Winder, W., Baldwin, K., and Holloszy, J. Exercise-induced increase in the capacity of rat skeletal muscle to oxidize ketones. Can. J. Physiol. Pharmacol. 53: 86-91, 1975.

Winer, B. Statistical Principles in Experimental Design. New York: McGraw-Hill, 1971.

Wise, W. Normal arterial blood gases and chemical components in the unanesthetized dog. J. Appl. Physiol. 35: 427-429, 1973.

APPENDIX A

REVIEW OF LITERATURE

FACTORS TRADITIONALLY ASSOCIATED WITH MUSCLE FATIGUE

In the investigation of the factors which limit muscle performance during repeated contractions, metabolic studies employing either isolated muscle preparations, exercising animals or humans have attributed the reversible muscle performance decrement to various metabolic occurrences. Each of these alterations in muscle metabolic state can be categorized into one of the four basic processes as outlined by Simonson (1971): accumulation of substances; depletion of substrates; changes in the physicochemical state of the substrate; or disturbance of regulation and coordination of metabolic processes.

Depletion of High-energy Phosphates (ATP, CP)

Adenosine triphosphate (ATP) is the source of the energy of muscle contraction (Lohmann, 1934). There are several metabolic means through which ATP is continually resynthesized: via oxidative phosphorylation in mitochondria; from ADP transphosphorylation of creatine phosphate (CP) through the enzyme creatine phosphokinase; from ADP with the formation of ATP and AMP through the enzyme myokinase; and via glycolysis (Lehninger, 1972).

In light of the importance of ATP as the ultimate energy source for contraction, many investigators have focussed their efforts towards attempting to correlate tissue ATP and CP concentrations with the onset of muscle fatigue. Hultman, et al. (1967) reported the first data on muscle ATP and CP concentrations in humans during exercise. Their results indicated a correlation between bicycle ergometer work load and CP concentration at the end of exercise, and illustrated a significant CP depletion (close to zero) and ATP reduction (to 40% of original

resting level) at the end of supramaximal exercise to exhaustion. Hultman et al. concluded that "the limiting factor in heavy work is the availability of energy-rich phosphate in muscle" (Hultman et al., 1967), and suggested reasons for this depletion during different intensities of exercise.

Gubjarnason et al. (1970) reported a "clear relationship" between contractility and CP levels in ischemic heart muscle, thus upholding the hypothesis of Hultman et al. (1967). These investigators expressed the belief that CP acts as an energy shuttle between the functional ATP compartments, and that myofibrillar ATP is not reflected in total tissue ATP levels, but rather by CP levels together with creatine phosphokinase activity. Spande and Schotteluis (1970), using mouse soleus muscle stimulated tetanically once every 20 seconds for three hours, reported that the isometric tension developed by the muscle was directly proportional to its CP concentration. These sources, in addition to a report by Karlsson et al. (1971) of a linear relationship between ATP and CP depletion and relative ergometer work load, tend to support the concept that there are measurable differences in the tissue concentrations of high-energy phosphates that can be associated with muscle fatigue.

On the other hand, evidence exists which tends to contradict this relationship. Karlsson (1971) reported that, in humans exercising on a bicycle ergometer above 90 to 100% of maximum oxygen uptake, quadriceps phosphagen (ATP and CP) stores were depleted to minimum values after 2 minutes of exercise, independent of whether or not the subject was exhausted, and underwent no further reduction with continued exercise. This same phenomenon occurred at work loads capable of exhausting the subjects in 2 to 3 minutes, 5 to 7 minutes, or 15 to 20

minutes (Karlsson and Saltin, 1970). Karlsson and Saltin (1971) illustrated that, during intermittent bicycle ergometer exercise bouts to exhaustion, quadriceps ATP and CP concentrations progressively declined over 5 bursts, even though fatigue occurred at each work bout. A similar lack of significant relationship between muscle exhaustion and muscle ATP and CP concentrations has been illustrated in cases where the contraction is isometric, at different intensities (Karlsson and Ollander, 1972).

In spite of the apparent contradiction, attempts to correlate high-energy phosphate concentrations and muscle exhaustion are necessarily limited by experimental factors. Suggestions that various functional ATP pools may exist in muscle (Gubjarnason et al., 1970; Edington et al., 1973) render questionable many conclusions based on measurements on whole tissue samples. In addition, the extreme rapidity of ATP resynthesis (Simonson, 1971; Lowry and Passonneau, 1972) contraindicates the use of time-consuming tissue extrication and freezing techniques which are characteristic of many of these studies.

Depletion of Muscle Glycogen

The importance of carbohydrate as an energy source for ATP resynthesis during contraction has been established by many investigators. Costill et al. (1973) has calculated, using respiratory quotients, that during a 30 km run 60 to 65% of the total energy requirements are derived from carbohydrate sources, and during a 10 mile run at 80% of maximal oxygen uptake, the contribution of carbohydrate to total energy expenditure may range from 67 to 87%. Carlson et al. (1971) reported, with humans exercising on a bicycle ergometer at 70% of maximum oxygen uptake, carbohydrate metabolism constituted 61% of the total exercise

oxygen uptake, with two-thirds of the carbohydrate oxidation attributable to glycogen breakdown. In the *in situ* dog gastrocnemius muscle, DiPrampero *et al*. (1969) estimated that, in working muscle requiring 5 times the resting metabolic rate, 52% of the exercise oxygen uptake was used for carbohydrate oxidation, while at 10 times resting metabolic rate, the metabolic contribution of carbohydrates was 62%. They also concluded from their arteriovenous data that most of the carbohydrate oxidized was from endogenous sources. Similarly, Hirche *et al*. (1970) reported a 60% contribution of muscle glycogen catabolism to the total energy requirements in dog gastrocnemius working at 80% of maximum oxygen uptake, while Chapler and Stainsby (1968) suggest that dog gastrocnemius working at 20 to 40 times resting metabolic rate utilizes 50 to 90% of the exercise oxygen uptake for the purpose of carbohydrate metabolism.

In spite of the obvious importance of muscle glycogen in the continuation of prolonged exercise, estimates of muscle glycogen utilization based on quantitative measurements of whole tissue pieces have yielded contradictory results. Carlson *et al*. (1971) and Ahlborg *et al*. (1967) have suggested that muscle glycogen depletion limits performance at 60 to 70% of maximum aerobic capacity, based on positive correlation between bicycle ergometer performance time to fatigue and initial muscle glycogen content, and between muscle glycogen depletion and performance time. Other authors have arrived at similar conclusions based on muscle glycogen depletion profiles in humans (Saltin and Karlsson, 1971; Taylor *et al*., 1971; Hultman and Bergstrom, 1973), animals (Terjung *et al*., 1970), and isolated muscle (Hirche *et al*., 1970) working at this approximate intensity level. Saltin and Karlsson (1971) hypothesized

that, between 65 and 89% of maximum oxygen uptake, muscle glycogen seems to be the limiting factor for prolonged performance. They suggested that, at metabolic rates greater than this, muscle glycogen depletion is not a factor in limiting performance, but the accumulation of anaerobic metabolites may contribute to fatigue.

There are reports, however, based on whole tissue concentration, that have contradicted this proposed relationship between muscle glycogen depletion and fatigue. Chapler and Stainsby (1968) reported no more than a 75% depletion of glycogen in stimulated dog gastrocnemius even at the onset of fatigue, and Stumfhauser and Lamb (1973) showed a 60% depletion in fatigued frog sartorius. Costill et al. (1971), studying humans performing exhaustive 10 mile runs on 3 successive days, found that on the third day subjects were able to run the 10 mile route with pre-exercise quadriceps glycogen concentrations which were lower than those measured at the end of the first exhausting run, and thus concluded that muscle glycogen depletion did not cause fatigue in this case. In another investigation, Costill et al. (1971) reported substantial quantities of glycogen remaining in the vastus lateralis, gastrocnemius and soleus muscles of fatigued subjects following a 10 mile run, thereby substantiating their claim that "glycogen depletion is an unlikely explanation for the fatigue experienced by the subjects in this investigation" (Costill et al., 1971). These results were found in spite of the fact that the metabolic rates of the runners were maintained within the range suggested by Saltin and Karlsson (1971) to be dependent on muscle glycogen concentration.

More recently, research into the heterogeneity of muscle fibres, relative to contraction speed and metabolic profile (Peter et al., 1973),

and the role of this heterogeneity exercise, has yielded more specific information regarding the utilization of muscle fibre glycogen during various intensities and durations of work. Using the PAS staining technique for glycogen (Pearse, 1969) it has been illustrated that it is possible to demonstrate the relative glycogen content of individual muscle fibres, provided that the muscle glycogen concentration does not exceed 80 mM glucose units per kg (Piehl, 1974). Using this stain in conjunction with stains for myosin ATPase (speed of contraction) and an oxidative enzyme such as NADH-diaphorase (oxidative potential)(Peter et al., 1972) glycogen depletion patterns in individual fibre populations have been demonstrated which are specific to the type and duration of exercise. Electrical stimulation of the muscle belly or nerve trunk in an isolated muscle preparation has been shown to elicit a rapid glycogen utilization in fast twitch fibres (Kugelberg and Edstrom, 1968; Edgerton et al., 1970). This heavy recruitment of fast twitch fibres, as evidenced by a decrease in PAS staining intensity in these fibres compared to slow twitch fibres has also been illustrated in vivo exercising muscle as a result of high intensity work (Gollnick et al., 1973; Armstrong et al., 1974; Piehl, 1974), rhythmic isometric contractions above 20% of maximum voluntary contraction (Gollnick et al., 1974) and at the end of prolonged exercise, at intensities up to maximum oxygen uptake, after the majority of slow twitch fibres have been depleted of glycogen (Gollnick et al., 1973; Piehl et al., 1974). However, glycogen depletion patterns suggesting recruitment of primarily slow twitch fibres has been illustrated in working muscles during exercise at submaximal intensities maintained for prolonged durations (Gollnick et al., 1973; Piehl, 1974) and rhythmic isometric contractions of less

than 20% MVC (Gollnick et al., 1974). It has been suggested that the selective recruitment of fibres during various types and intensities of exercise may be a consequence of differences in activation threshold and/or frequency threshold between slow twitch and fast twitch fibres (Henneman and Olson, 1965; Piehl, 1974). Whatever the basis for the selective recruitment, the absence of PAS reaction in specific muscle fibre populations resulting from various types of and durations of exercise lends credence to the hypothesis that muscle fatigue may be localized to that population of fibres being recruited for the activity.

At present, a number of conditions limit conclusions that can be made regarding muscle glycogen depletion and fatigue. The validity of using the muscle biopsy technique has been questioned by Chapler and Moore (1972), who have illustrated the lack of homogeneity of muscle glycogen profiles when samples are excised from different portions of the same dog gastrocnemius muscle. In addition, the procedures for determining glycogen content in individual fibres is as yet semi-quantitative. Finally, changes in the activities of enzymes involved in glycogen metabolism during prolonged exercise, such as the phosphorylase (Danforth and Helmreich, 1964) and synthetase (Taylor et al., 1972) systems, may limit the products of glycogen breakdown, and thus enhance the onset of fatigue, while still maintaining muscle glycogen concentrations undepleted.

Availability of Oxygen

Evidence to date suggests that the supply of oxygen during exercise in a normoxic environment appears to be adequate enough to be disregarded as a factor limiting performance. Even during maximal high-intensity work, the femoral venous oxygen pressure in the exercising

human decreases to 21.7 mmHg (Keul et al., 1964) which is still considerably higher than the critical venous pressure of 10 mmHg cited by Stainsby and Otis (1964), below which muscle begins to show signs of fatigue due to inadequate oxygen supply. In an investigation utilizing dog gastrocnemius muscle contracting at 5 twitches per second (20 to 40 times resting metabolic rate), Jobsis and Stainsby (1968) concluded that oxygen was in plentiful supply to the muscle. By following the oxidation-reduction level of mitochondrial pyridine nucleotides fluorometrically, they found that NADH consistently became oxidized, in spite of lactate production by the muscle. Jobsis and Stainsby (1968) reported a similar tendency towards the further oxidation of intramitochondrial NADH when glycolysis was impaired by administering iodoacetate, thus suggesting that substrate supply is limiting in fatiguing exercise. The only case in which they found a tendency towards the further reduction of intramitochondrial NADH was when oxygen supply to the muscle was eliminated, and when Amytal (amobarbital), which blocks oxidative phosphorylation was administered.

Stainsby and Otis (1964), using the in situ dog gastrocnemius preparation, reported that the work profiles and oxygen uptake of a muscle working at close to maximum aerobic capacity were constant, in spite of blood oxygen tensions altered above and below normal, until a critical arterial pO_2 of 40 mmHg was attained. Below this oxygen content, effects were noticed on oxygen consumption and tension capabilities. Based on this evidence, Stainsby (1973) has concluded that "the rate limiting processes would appear to reside within the muscle, probably in the excitation - contraction coupling or contractile apparatus". These conclusions have been bolstered further by research evidence from

studies of prolonged exercise under conditions of hyperbaric conditions (Wilson et al., 1975), and the reported high degree of correlation between maximum oxygen consumption and muscle mitochondrial dimensions in humans (Hoppeler et al., 1973).

Muscle Lactate Accumulation and pH Change

Review of the literature indicates that the accumulation of lactate in muscle tissue, and the concomitant pH decrease which accompanies lactate production, may play a significant role in limiting the contractile capabilities of the working muscle. Karlsson and Saltin (1970) reported similarly high muscle lactate levels in the quadriceps muscles of men working to exhaustion in 2 to 3 minutes, and 5 to 7 minutes, and suggested muscle lactate accumulation as a limiting factor at these work loads. In a later investigation, similar results were reported from men performing exhausting intermittent exercise bouts on the bicycle ergometer (Karlsson and Saltin, 1971). Hirche et al. (1975) have shown in isolated dog gastrocnemius that lactate can accumulate in muscle during high intensity contractions and that alterations in the pH of the blood perfusing the working muscle can increase the removal of the muscle-accumulated lactate, and can decelerate the normal rate of fatigue of the muscle, in parallel. Their results suggest that the muscle membrane may constitute a barrier to the non-ionized form of lactate, and that an increased pH of perfusing blood allows more efficient lactate removal via an exchange mechanism involving bicarbonate and lactate ions.

There have been numerous mechanims proposed by which lactate production in muscle tissue and the concomitant pH decrease may affect contraction. Although mitochondrial respiration has been shown to be

relatively insensitive to large changes in intracellular pH (Tobin et al., 1972; Mitchelson and Hird, 1973), other pH sensitive systems in muscle include glycolysis at the level of phosphofructokinase (Trivedi and Danforth, 1966; Bergstrom et al., 1973), the phosphorylase system (Hultman and Bergstrom, 1973), the ATPase activity of the Ca^{++} pump (Gonzalez-Serratos et al., 1974), and the kinetics of the creatine phosphokinase reaction (Sahlin et al., 1975). Katz (1970) has proposed that, in acidotic heart muscle, hydrogen ions may compete with Ca^{++} for the troponin binding sites, thereby decreasing muscle contractile capability. Fuchs (1970) has examined this competitive effect in vitro, using pH ranges comparable to those seen in exercising muscle (Hermansen and Osnes, 1972), thus presenting further evidence for a possible mechanism by which the production of lactate in muscle tissue may possibly interfere with contractility.

However, it appears that lactate accumulation in working muscle does not play a major role in muscle fatigue in all types and intensities of exercise. It is a well known fact that lactate production is minimal near the end of prolonged bouts of cross-country skiing (Astrand et al., 1963) and running (Costill et al., 1973), in spite of fatigue. Taylor and Rao (1973) reported muscle lactate levels in rats which had performed prolonged runs to exhaustion that were not significantly different from resting rats. Similar results were reported by Terjung et al. (1972). Dawson et al. (1971) reported that muscle and blood lactate values were different for rats swum to fatigue in water at different temperatures. In the in situ dog gastrocnemius muscle, Welch and Stainsby (1967) have illustrated that, as the oxygen uptake and tension development of the working muscle decreased as it

progressed towards fatigue, lactate production was very low. Vesell and Pool (1966) have shown that, in canine muscle stimulated anaerobically to exhaustion, the muscle is capable of working at muscle lactate concentrations that are much higher than those seen during exercise or normal stimulation.

Evidence from human studies in which the subjects were performing high intensity work of both an isotonic (Karlsson et al., 1971)and isometric (Karlsson and Ollander, 1972; Gollnick et al., 1974) nature suggest that muscle lactate levels vary considerably, depending on the time and intensity of effort, thereby raising questions as to the role of lactate in affecting muscle performance, even during this type of work where lactate production is high.

Alterations in Muscle Temperature

Saltin et al. (1972) investigated various body temperatures in humans exercising to exhaustion, on a bicycle ergometer, at different ambient temperature conditions. From their results they concluded that "the one internal body temperature with a consistent value at the end of exhaustive exercise is a muscle temperature of around 40°C, which temperature could conceivably be a key factor that determines the limit of performance" (Saltin et al., 1972). Edwards et al. (1972) reported that the energy supply from anaerobic sources increased with increasing temperature in human quadriceps muscle contracted to two-thirds of maximum voluntary contraction to exhaustion in water baths of different temperature, and suggested that fatigue in this case was temperature-dependent.

Brooks et al. (1971) have reported a decrease in respiratory control in isolated rat muscle mitochondria between the temperatures of 37°C

and 45°C, and therefore a decrease in energy conserving efficiency. The work of Schafer (1973) suggests that fatigue in muscle may involve a phase transition in mitochondrial membrane lipids with increasing temperature, and thus an alteration in the activities of membrane-bound enzymes, which include those of the citric acid cycle and respiratory chain.

Muscle Electrolyte Alterations

Reported changes in muscle electrolyte concentrations with exercise have included decreased muscle potassium concentration (Ahlborg et al., 1967; Bergstrom et al., 1971), sodium accumulation (Ahlborg et al., 1967), a possible chloride shift into the cell (Bergstrom et al. 1971), and inorganic phosphate losses from working muscle (Hudlicka, 1973). It has been postulated that many of these apparent changes are due to the fluctuations in intracellular and extracellular fluid that occur during muscle contraction (Jacobsson and Kjellmer, 1964; Ahlborg et al., 1967; Bergstrom et al., 1973), and that muscle is still capable of contracting optimally in spite of these changes (Bergstrom et al., 1973).

Hudlicka (1973) upholds that muscle fatigue may be associated with muscle inorganic phosphate loss. This investigator reported a release of inorganic phosphate from cat gastrocnemius in which the external work decreased by 50%, wherease no release of inorganic phosphate was evident from cat soleus whose external work remained constant throughout the contraction period. The conclusion that "the presence of a sufficient amount of inorganic phosphate is a prerequisite for muscle performance without fatigue" (Hudlicka, 1973) was strengthened by the fact that fatiguing muscle regained its original work performance level

incubated in a medium containing increased amounts of inorganic phosphate in solution.

Cellular Disruption

Gollnick and King (1969) reported marked mitochondrial swelling, large spaces between adjacent myofibrils, and degenerated cristae in the gastrocnemius muscles of rats run to exhaustion. Swollen mitochondria in frog muscle fatigued via repeated tetani have also been reported by Gonzalez-Serratos et al. (1974). Dohm et al. (1973), in their study of the effect of hypoxia on the oxidative capacity of skeletal muscle in trained and untrained rats, suggested that the effects of hypoxia and exhaustive exercise may be mediated through a common factor; the activation of mitochondrial phospholipase by free fatty acids which would cause mitochondrial swelling and lowered oxidative capacity. In addition, Schafer (1973) has shown temperature-induced phase changes in mitochondrial membranes in vitro which may play a role during exercise.

However, mitochondria from muscles of rats run to exhaustion show no changes in respiratory cycle indices or P/O ratios, and no leakages of citric acid cycle enzymes (Terjung et al., 1972). In addition, Bowers et al. (1974) and Gale (1974) have independently reported no mitochondrial structural changes as a result of exhausting exercise in rats, using improved tissue fixative techniques. Both concluded that the mitochondrial changes previously reported may have been related to the sensitivity of the exhausted muscle tissue to certain fixative procedures and that no mitochondrial swelling or disruption occurs in vivo as a result of exhaustive exercise.

CELLULAR REDUCTION-OXIDATION STATE OF PYRIDINE NUCLEOTIDES

Nicotinamide adenine dinucleotide (NAD^+; diphosphopyridine nucleotide, DPN^+) is one of the three oxidation-reduction coenzymes (Lehninger, 1972). This coenzyme functions in conjunction with a class of dehydrogenases, termed pyridine nucleotide-dependent dehydrogenases, in the following manner:

$$\text{reduced substrate} + NAD^+ \rightleftharpoons \text{oxidized substrate} + NADH + H^+$$ (Sund, 1970)

The dehydrogenases which catalyze these reactions transfer reversibly two reducing equivalents from the substrate to the oxidized form of the pyridine nucleotide; one of these appears in the reduced pyridine nucleotide as a hydrogen atom, the other as an electron. The other hydrogen atom removed from the substrate appears as a free H^+ in the medium (Lehninger, 1972).

NAD^+ - NADH in Glycolysis

At one step in glycolysis, glyceraldehyde-3-phosphate is oxidized to phosphoglycerate. During this oxidation NAD^+ is converted to NADH:

$$\text{glyceraldehyde-3-phosphate} + NAD^+ + P_i \xrightarrow[\text{dehydrogenase}]{\text{glyceraldehyde-3-phosphate}} \text{phosphoglycerate} + NADH + H^+$$ (Lehninger, 1972)

The reduction of NAD^+ to NADH in the glyceraldehyde-3-phosphate dehydrogenase reaction requires that the NADH which is produced be reoxidized at the same rate as glycolysis, in order to maintain glycolytic flux (Newsholme and Start, 1973). This reoxidation of NADH can be accomplished in the muscle cell sarcoplasm via the lactate dehydrogenase reaction:

$$\text{pyruvic acid} + NADH \xrightleftharpoons[\text{dehydrogenase}]{\text{lactate}} \text{lactic acid} + NAD^+$$

The kinetics of this reaction strongly favour lactate production (Newsholme and Start, 1973), but may vary according to the isozyme pattern, especially relative to the contractile speed and metabolic characteristics of individual fibres (Karlsson et al., 1974).

Shuttle Mechanisms for Hydrogen Electrons Generated in Glycolysis

The hydrogen electrons generated by the glyceraldehyde-3-phosphate dehydrogenase reaction are capable of utilization as substrate in the electron transport system in mitochondria. However, since NAD^+ and NADH are impermeable to the mitochondrial membrane (Purvis and Lowenstein, 1961), hydrogen electrons must be transported from the reduced NADH in the cytoplasm to the oxidized form in the mitochondria by various "shuttle mechanisms" (Boxer and Devlin, 1961). In fibres containing high glycolytic capacity, cytoplasmic NADH can be reoxidized, and the reducing equivalents transported to the mitochondrial NAD^+, via the α-glycerophosphate-dihydroxyacetone phosphate shuttle (Peter et al., 1972), as depicted in Figure 9. In fibres with high oxidative capacity, the malate-aspartate shuttle (Figure 10) appears to predominate (Newsholme and Start, 1973).

NAD^+ and NADH in Mitochondrial Electron Transport

In the citric acid cycle, pyridine nucleotide-linked dehydrogenases operate to oxidize substrates and reduce NAD^+, at the level of isocitrate, α-ketoglutarate, and malate oxidation (Lehninger, 1972). The electrons carried by NADH are removed and transferred, via a large free-energy change, to molecular oxygen by a series of oxidoreductive intermediates known collectively as the electron transport chain (Lehninger, 1972). At three distinct sites along the chain, the free-energy change is sufficient to allow coupling with the phosphorylation of ADP to ATP.

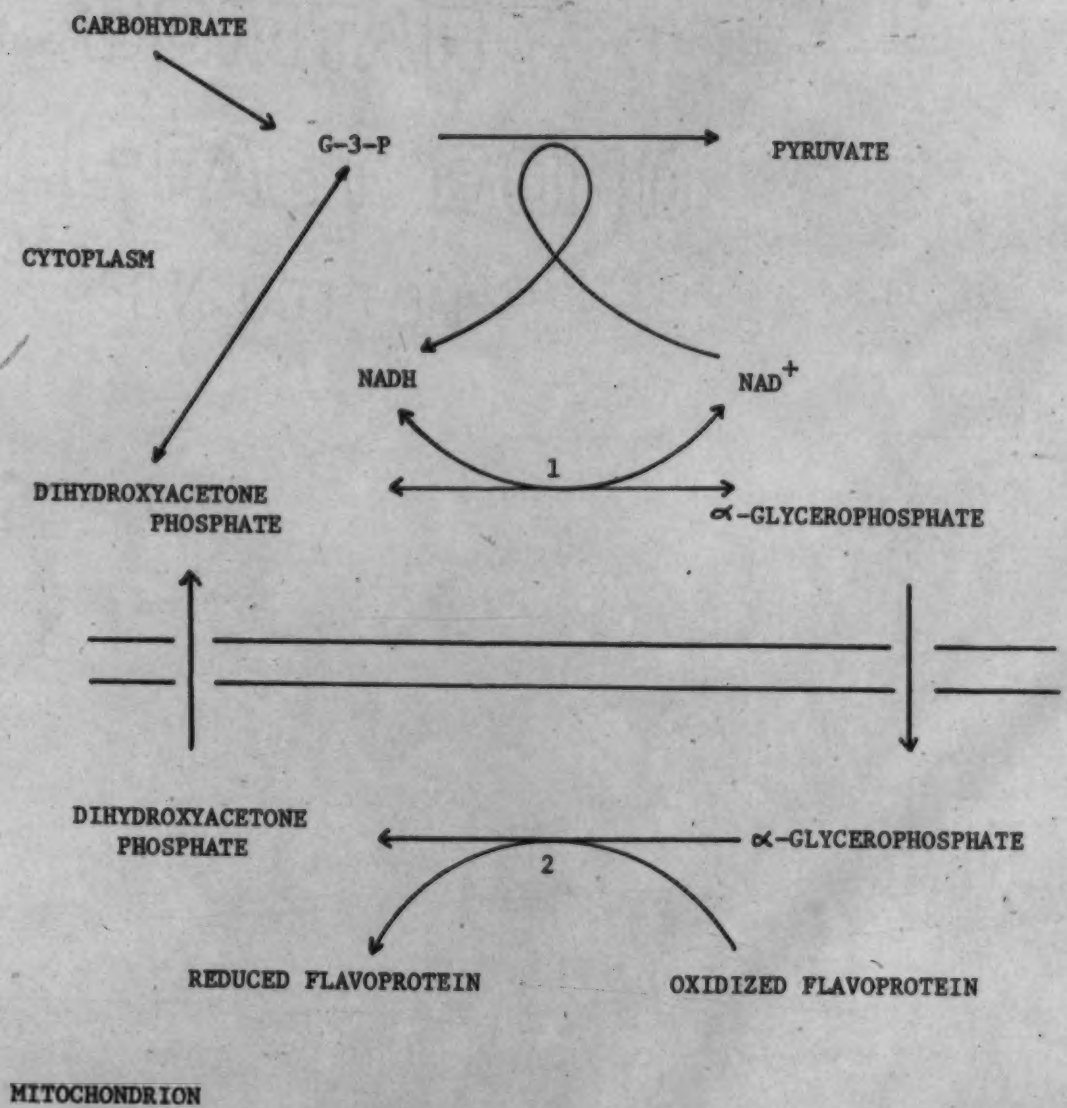


Figure 9: The ∝-Glycerophosphate-Dihydroxyacetone Phosphate Shuttle. (1) Cytoplasmic ∝-glycerophosphate dehydrogenase; (2) Mitochondrial ∝-glycerophosphate dehydrogenase (Newsholme and Starte, 1973)

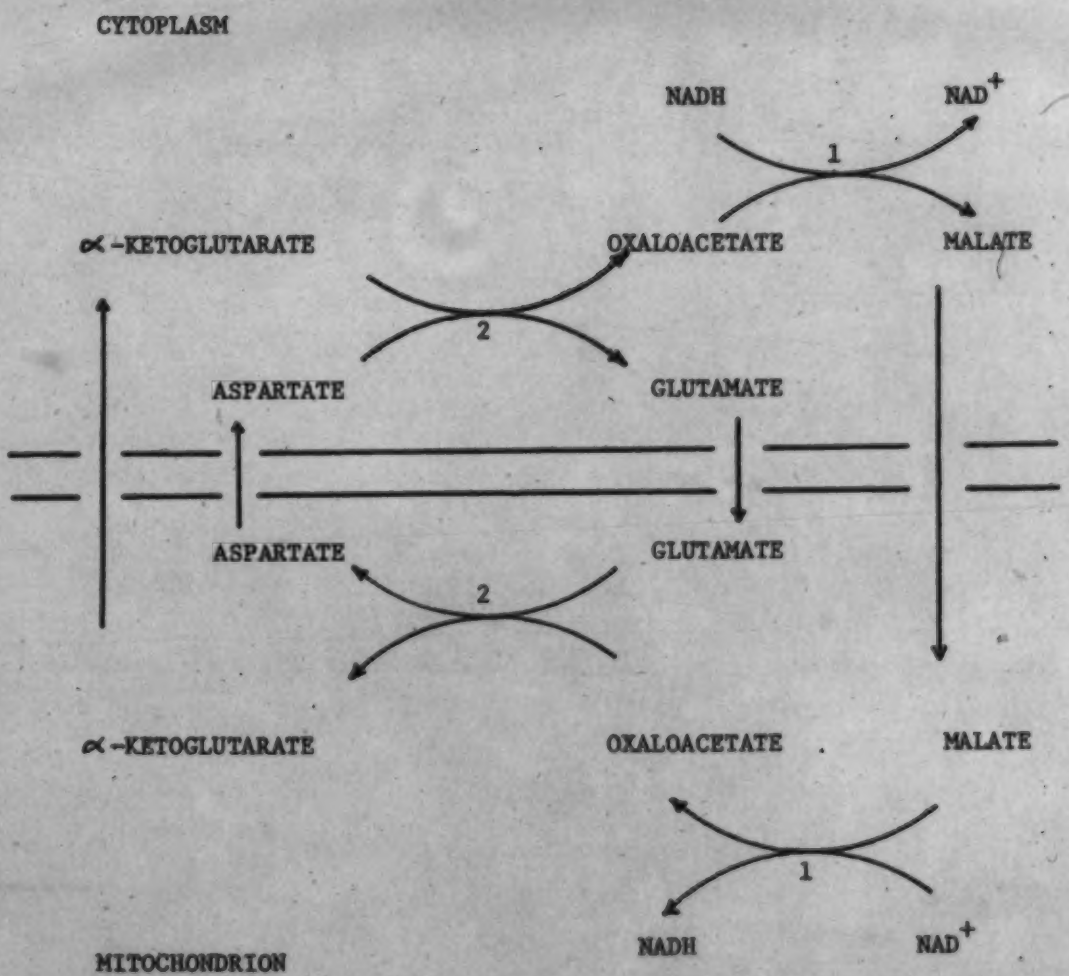


Figure 10: The Malate-Aspartate Shuttle. (1) Malate dehydrogenase; (2) Glutamate-oxaloacetate transaminase (Newsholme and Starte, 1973)

Mitochondrial and Cytoplasmic Redox States

Measurements of total NAD^+ and NADH in respiring tissue do not provide valid information about the concentrations of pyridine nucleotides which are available to the various dehydrogenase systems located in specific cellular compartments (Newsholme and Start, 1973). Although whole tissue measurements give an indication of the general redox state of the entire cell, they do not take into consideration the established fact that the mitochondrial and cytoplasmic compartments differ markedly in their redox states (Krebs et al., 1962; Krebs, 1973). Williamson, Lund and Krebs (1969) have reported $[NAD^+]/[NADH]$ ratios of 200 to 1000 in the cytoplasmic compartment of rat liver cells, and mitochondrial $[NAD^+]/[NADH]$ ratios of 2 to 10, both of which can vary in altered metabolic states. Similar values have been reported for muscle tissue (Edington et al., 1973).

The difference between the redox states of pyridine nucleotides in different compartments of the same cell has metabolic significance, and is based on the equilibria of the pyridine nucleotide-dependent dehydrogenases. The cytoplasmic glyceraldehyde-3-phosphate dehydrogenase catalyses a reaction which is close to equilibrium. Therefore, the flux through this reaction depends upon the ratio of reactants and products (Newsholme and Start, 1973). Since glyceraldehyde-3-phosphate, the principle substrate, is kept in low concentration due to its equilibria with dihydroxyacetone phosphate and fructose diphosphate, its low concentration limits the flux through this reaction in the direction of glycolysis unless the $[NAD^+]/[NADH]$ ratio is high (Newsholme and Start, 1973), thereby necessitating a high cytoplasmic $[NAD^+]/[NADH]$ ratio if glycolysis is desired.

However, in the mitochondria the $[NAD^+]/[NADH]$ system must be maintained at a much more reduced level in order to provide sufficient reducing power to drive electrons along the electron transport chain for the generation of ATP (Newsholme and Start, 1973). To be effective as an energy source, the mitochondrial $[NAD^+]/[NADH]$ ratio must be below a critical value, if the free-energy change of the transfer of electrons from NADH to flavoprotein is to be large enough for coupling with the synthesis of ATP (Krebs, 1973).

Thus in order to maintain a satisfactory rate of glycolysis in the cytoplasm and a satisfactory rate of electron transport in mitochondria, a large difference between the $[NAD^+]/[NADH]$ ratios in the two compartments is required. This is facilitated by the impermeability of the mitochondrial membrane to pyridine nucleotides (Lehninger, 1972), and the presence of metabolic shuttles for hydrogen electrons (Boxer and Devlin, 1961). In addition, these shuttles must be non-equilibrium processes if equilibrium of the two redox pools on either side of the mitochondrial membrane is to be avoided (Newsholme and Start, 1973).

The Significance of Cellular Redox States

The intracellular oxidation-reduction potential (redox state) is a fundamental physicochemical property of biological systems, influencing the chemical behaviors of all oxidizable or reducible compounds in the system (Mintz and Robin, 1971). Since NAD^+ and NADH are of major metabolic significance, as has been illustrated above, the redox state of this particular pair may prove important in assessment of possible mechanisms of muscle fatigue.

Krebs (1973) points out that measurement of the redox state of NAD^+/NADH system is of significance in metabolic studies in that:

1. The redox state determines the direction of reversible reactions;

2. The redox state determines the extent to which pyridine nucleotides can be effective as reducing agents;

3. The value of the ratio determines the magnitude of free-energy changes of oxido-reductions such as those accompanying the transport of electrons from NADH to flavoproteins in the electron transport chain. Unless the free energy changes of these reactions are above a critical minimum, there can be no effective coupling with the synthesis of ATP (Krebs, 1973).

Although the result of changing metabolic conditions on cellular redox states was first observed in rat liver (Williamson et al., 1969; Krebs, 1973), similar studies have been performed on brain (Folbergrova et al., 1972), alveolar macrophages (Mintz and Robin, 1971), cardiac muscle (Kraupp et al., 1967), renal cortex (Hemms and Gaja, 1972), adipose tissue (Krebs, 1973), and skeletal muscle (Edington et al., 1973). In all cases, the redox states of NAD^+/NADH system in the cytoplasmic and mitochondrial compartments have been observed to be similar to those observed in liver.

In interpreting the meaning of redox state measurements, it appears that the redox state of $[NAD^+]/[NADH]$ is controlled by the state of phosphorylation of the adenine nucleotides (ATP, ADP, AMP). The work of Krebs et al. (1962, 1969, 1973) has revealed that the ratio $[ATP]/[ADP + Pi]$ is directly proportional to the $[NAD^+]/[NADH]$ ratio in the cytoplasm, and inversely proportional in the mitochondria. In muscle tissue during such a violent change in metabolic state as exercise sustained to fatigue, measurements of $[NAD^+]/[NADH]$ ratios would

give an indication of relative states of phosphorylation of adenine nucleotides in the mitochondria, where ATP is being formed at a near maximal rate, and in the cytoplasm, where it is being used as the primary fuel for muscle contraction. Also, these measurements would give indications as to the cellular sites at which substrate oxidation might be inhibited due to changes in this redox state, i.e., decreased availability of NAD^+ for continued glycolytic flux in cytoplasm, or decreased NADH for maximum respiration in mitochondria. This approach appears to be an attractive supplement to the assessment of muscle fatigue on the basis of individual substrate or enzyme systems.

The Measurement of Cellular Redox State

In the estimate of the redox state of pyridine nucleotides in cytoplasm and mitochondria, direct measurements of total cell NAD^+ and NADH are not appropriate, since, while both NAD^+ and NADH exist in the cell in protein-bound form, only free unbound nucleotides are directly involved in oxidation-reduction potential (Mintz and Robin, 1971). In addition, since separate cytoplasmic and mitochondrial compartments of NAD^+ and NADH have been reported (Williamson et al., 1969; Krebs, 1973), measurement of total cell nucelotides cannot provide precise information on the redox state in each compartment. This problem cannot be dealt with by usual methods of tissue fractionation because the redox state of the nucleotides is liable to undergo rapid changes during the process of fractionation (Williamson et al., 1969).

It has been previously proposed that these difficulties may be resolved by using metabolic reactions localized in the cytoplasm and mitochondria which are NAD^+ -NADH linked and are also close to equilibrium ("redox couples") (Williamson et al., 1969). For example, the

conversion of lactate to pyruvate has been shown to be essentially cytoplasmic in location (Mintz and Robin, 1971), while the conversion of β-hydroxybutyrate to acetoacetate is localized in the mitochondrial cristae (Lehninger et al., 1960). From consideration of mass action law, theoretically one can calculate the free $[NAD^+]/[NADH]$ ratios for each compartment, as shown below (Newsholme et al., 1973).

for cytoplasm:

$$\frac{[NAD^+]}{[NADH]} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \times \frac{1}{K_{LDH}}$$

for mitochondria:

$$\frac{[NAD^+]}{[NADH]} = \frac{[\text{acetoacetate}]}{[\beta\text{-hydroxybutyrate}]} \times \frac{1}{K_{HBD}}$$

where:

K_{LDH} = equilibrium constant for the reaction catalyzed by lactate hydrogenase

K_{HBD} = equilibrium constant for the reaction catalyzed by β-hydroxybutyrate dehydrogenase

Therefore, since the substances taking part in these reactions are freely diffusible over the mitochondrial membrane, one can make measurements on whole tissue to yield estimates of redox states of NAD^+-NADH in separate cell compartments.

Muscle Metabolism and $[NAD^+]/[NADH]$ Ratios

Jobsis and Stainsby (1968) were the first to report whole muscle $[NAD^+]/[NADH]$ fluctuations in working muscle. They found that, when they examined the oxidation-reduction level of intracellular NAD fluorimetrically in dog muscle contracting at near maximum metabolic rates, NADH went consistently oxidized. They thus concluded that, in these muscles, the oxygen supply never limited respiratory chain activity

during contractions.

Edington (1970), examining total muscle [NAD^+]/[NADH] and [$NADP^+$]/[NADPH] in contracting rat gastrocnemius-plantaris muscle, reported that the greater the work performance of the muscle during ten minutes of submaximal contraction the more capable the cell was of tolerating a more reduced state. Furthermore, he found that, for any given work performance level, the muscle from a trained animal had a higher oxidized-to-reduced ratio than the muscle from a non-trained animal.

Chagovets (1972) reported a decrease, below resting values, in the [NAD^+]/[NADH] ratio in hind limbs of Wistar rats swum for 15 minutes.

The only reported experimental data on compartmental redox states in exercising muscle are from research by Edington, Ward and Saville (1973). In their investigation, they estimated cytoplasmic and mitochondrial [NAD^+]/[NADH] ratios, using redox couples, in rat gastrocnemius plantaris muscle contracted for 10 minutes at a submaximal metabolic rate. The work output data showed no indication of muscle fatigue. In nontrained rats, the cytoplasmic [NAD^+]/[NADH] ratio increased from 300 at rest to 1800 after 30 seconds of contraction, and then returned to resting levels by 10 minutes of work; in trained rats, the value increased, at a faster rate, to 3000 by 30 seconds, and continued to 4200 by 10 minutes. From this, they concluded that in the trained muscle, glycolysis was enhanced due to an increasing availability of NAD^+. In mitochondria, the [NAD^+]/[NADH] ratio for nontrained muscle increased from one at rest to 48 at 30 seconds of work and subsequently decreased to 24 by 10 minutes; in trained rats, the ratio increased only to 14 at 30 seconds and decreased again to 7 by 10 minutes. From this Edington et al. (1973) concluded that, in the mitochondria, the supply of

reducing equivalents was limited, but that this supply was not as limited in trained as in nontrained muscle, as witnessed by the low [NAD^+]/[NADH] ratios during exercise.

APPENDIX B

BURGER BITS

(Standard Brands, Montreal)

Guaranteed Analysis

Crude Protein	Not less than 23%
Crude Fat	Not less than 7%
Crude Fiber	Not more than 5%
Ash	Not more than 10%
Moisure	Not more than 12%
Calcium	Min 1%, Max 2%
Salt (NaCl)	Min 1%, Max 1.8%

Ingredients

Ground corn, wheat red dog, meat and bone meal, soybean meal, wheat middlings, animal fat - preserved with B.H.T., beef hydrolysate, dried beet pulp and/or tomato pomace, dried whey product, trace mineralized salt, soybean oil potassium chloride, iron oxide, vitamin B-12 supplement, vitamin A and E supplement, vitamin D supplement.

TISSUE LACTATE

PILOT EXPERIMENT

(August, 1974)

Freeze-clamped muscle plugs were taken simultaneously from the left and right gastrocnemius muscle groups, both of which were similarly isolated in terms of nervous innervation and venous drainage. Measurements of tissue lactate were performed in duplicate on each tissue sample. Tissue lactate values were 1.67 (left muscle) and 1.56 (right muscle) μM/g wet weight of muscle. This is within the range of normal resting lactate concentration reported from this muscle preparation (Corsi et al., 1969; Hirche et al., 1973).

APPENDIX C

PLATE I X-RAY OF ISOLATED BLOOD SUPPLY OF GASTROCNEMIUS-FLEXOR MUSCLE GROUP, SHOWN AFTER INJECTION OF 30% HYPAQUE

PLATE IIA SURGICALLY ISOLATED GASTROCNEMIUS-FLEXOR MUSCLE GROUP

PLATE IIB ISOLATED MUSCLE GROUP WITH FEMUR FIXED AND VENOUS OUTFLOW CATHETER IN PLACE

PLATE IIIA CALCANEUS ATTACHED TO MYOGRAPH-LINEAR DISPLACEMENT TRANSDUCER COMPLEX

PLATE IIIB FLOW PROBE AND VENOUS BLOOD SAMPLING PORT

PLATE IVA MOUNTED GASTROCNEMIUS-FLEXOR MUSCLE GROUP PREPARATION

PLATE IVB MOUNTED MUSCLE GROUP WITH STIMULATING ELECTRODE, RINGERS DRIP AND THERMOCOUPLE IN PLACE

APPENDIX D

CALCULATION OF OXYGEN CONSUMPTION

(Davidsohn and Henry, 1969)

1. Determine % O_2 saturation of arterial and venous hemoglobin from pO_2 and pH, using nomogram of Astrup et al. (1965).

2. Determine O_2 capacity (ml/ml) of arterial and venous blood by:

$$\frac{\text{hemoglobin (g\%)} \times 1.36}{100}$$

3. Determine O_2 content (ml/ml) of arterial and venous blood by:

O_2 content = % O_2 saturation X O_2 capacity

4. Determine O_2 consumption (ml/min) by:

(O_2 content_A - O_2 content_V) X blood flow (ml/min)

MEASUREMENT OF MUSCLE ARTERIOVENOUS DIFFERENCES: CORRECTION FOR PLASMA WATER LOSS

(Schlein et al., 1973)

This calculation is based on the Fick principle (Rushner, 1970), with correction for the uptake or release of plasma water by the working muscle.

$(flow_a \times [S]_a) - (flow_v \times [S]_v)$ = net uptake or release of substance S

where:

$flow_v$ (venous plasma flow) = venous blood flow X (1-hematocrit)

$flow_a$ (arterial plasma flow) = $flow_v \times Tp_V/Tp_A$

TP_V = total protein concentration in venous plasma

TP_A = total protein concentration in arterial plasma

ESTIMATION OF CYTOPLASMIC AND MITOCHONDRIAL [NAD+]/[NADH] RATIOS

(Williamson et al., 1969)

Cytoplasm: $$\frac{[NAD^+]}{[NADH]} = \frac{1}{K_{LDH}} \times \frac{[pyruvate]}{[lactate]}$$

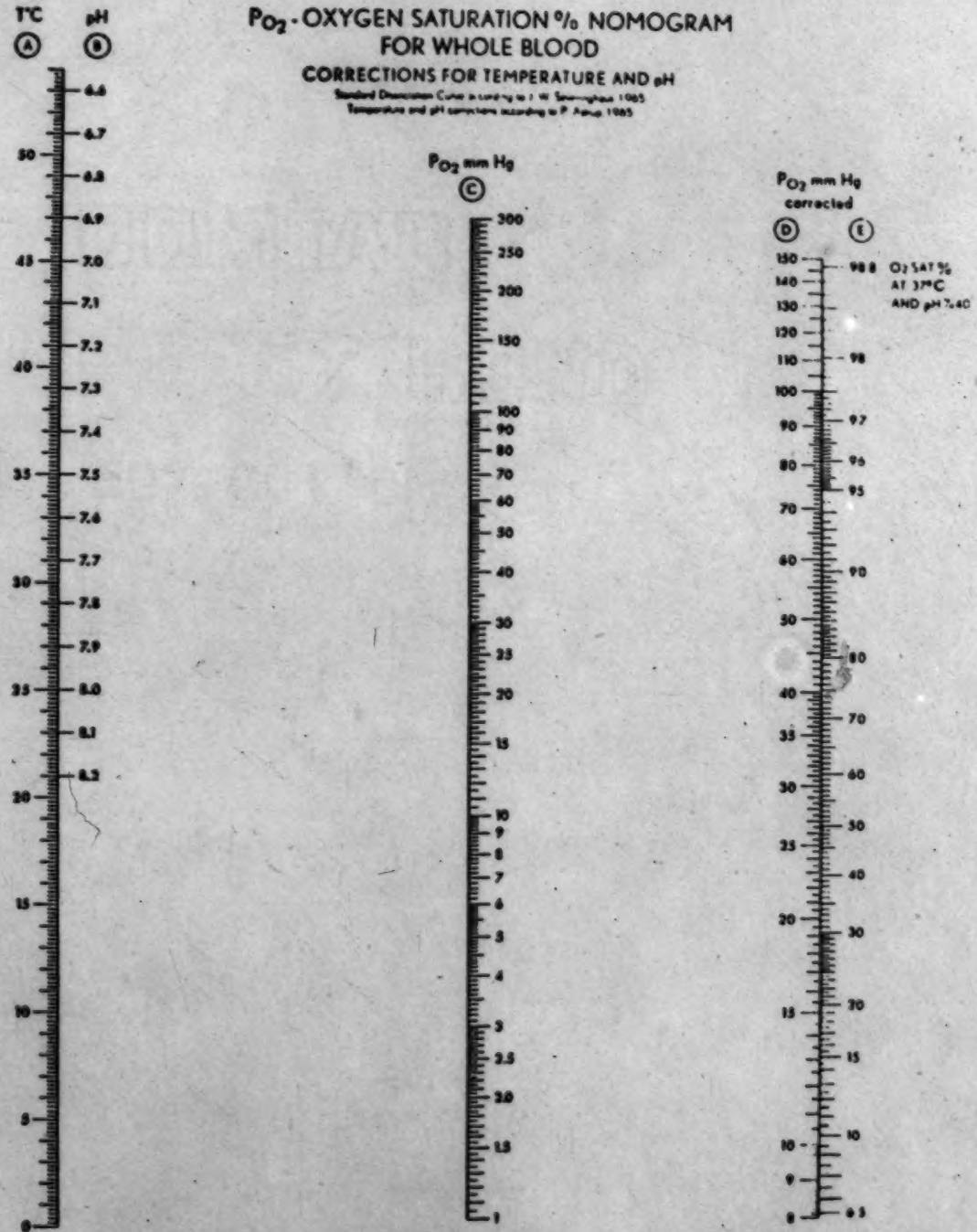
where: $K_{LDH} = 1.11 \times 10^{-4}$

Mitochondria: $$\frac{[NAD^+]}{[NADH]} = \frac{1}{K_{BDH}} \times \frac{[acetoacetate]}{[\beta\text{-hydroxybutyrate}]}$$

where: $K_{BDH} = 4.93 \times 10^{-2}$

Assumptions (Williamson et al., 1969)

1. The pH of mitochondria and cytoplasm is 7.0.
2. The ionic strength of both compartments is 0.25.
3. Both reactions are at equilibrium.



Clinical Diagnosis by Lab. Methods

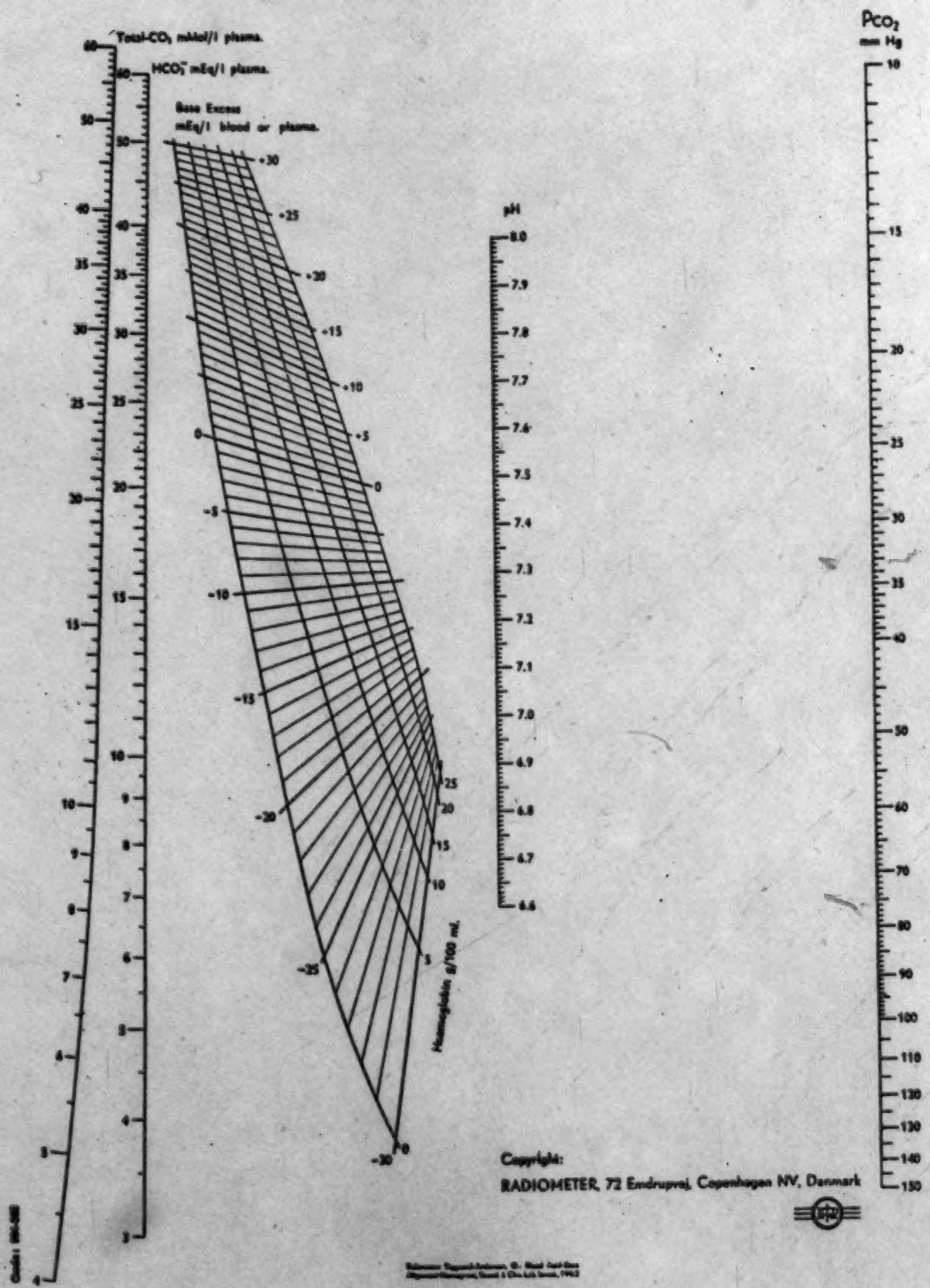
Todd - Sanford Davidson & Henry page 667

Saunders - Philadelphia, London, Toronto

Radiometer

Scand. J.Clin. Lab. Invest.
17: 515, 1965.

SIGGAARD-ANDERSEN ALIGNMENT NOMOGRAM



Copyright:
RADIOMETER, 72 Emdrupvej, Copenhagen NV, Denmark

APPENDIX E

EXPERIMENT NUMBER	DOG WEIGHT (KG)	BREED (HIGHEST PROPORTION)	AGE	ASSIGNED CONDITION	SEX
9	27.5	German Shepherd	2 yrs	50%L	M
10	15.0	Spaniel	2 yrs	50%L	M
11	20.0	German Shepherd	1 yr	50%L	F
12	18.0	Collie	10 mos	80%	F
13	19.0	German Shepherd	1 yr	50%	M
14	15.5	Poodle	2 yrs	50%	M
15	18.5	German Shepherd	2 yrs	65%	F
16	14.8	Collie	2 yrs	65%L	F
17	17.5	Collie	4 yrs	65%	F
18	21.3	Collie	2 yrs	65%	M
19	23.5	Labrador	1 yr	65%L	F
20	13.5	Samoyed	2 yrs	65%L	M
21	18.0	Collie	1 yr	80%	F
22	23.5	Samoyed	2 yrs	80%	F
23	15.2	Collie	2 yrs	50%	M

CONT'D

EXPERIMENT NUMBER	DOG WEIGHT (KG)	BREED (HIGHEST PROPORTION)	AGE	ASSIGNED CONDITION	SEX
24	17.5	Collie	2 yrs	50%L	M
26	15.5	Collie	1 yr	50%	M
27	19.5	German Shepherd	2 yrs	80%	F
28	16.2	Setter	1 yr	50%L	M
29	23.3	German Shepherd	2 yrs	65%	F
30	22.5	Collie	2 yrs	80%	M
31	23.0	Collie	1 yr	50%L	M
32	19.5	German Shepherd	1 yr	65%	M
33	12.0	Spaniel	1 yr	65%L	F
34	18.2	German Shepherd	5 yrs	65%L	F
35	19.4	Labrador	1 yr	50%	F
36	20.7	German Shepherd	3 yrs	50%	M
37	27.5	German Shepherd	5 yrs	50%	F
38	16.5	Collie	1 yr	65%	M

TABLE XVI BODY WEIGHTS, BREED, AGE, SEX, AND EXPERIMENTAL CONDITION OF THE ANIMALS INVOLVED IN THE STUDY

PARAMETER	BEGINNING OF STIMULATION	END OF STIMULATION
Arterial blood pO_2 (mm Hg)	99.1 ± 2.5	95.2 ± 3.8
Arterial blood pCO_2 (mm Hg)	35.9 ± 1.8	39.7 ± 1.8**
Arterial blood pH	7.30 ± 0.01	7.24 ± 0.01**
Arterial blood hemoglobin (g%)	13.7 ± 0.7	12.8 ± 0.7
Arterial blood hematocrit (%)	40.5 ± 1.9	37.6 ± 2.1*
Systolic blood pressure (mm Hg)	98 ± 3	93 ± 3*
Diastolic blood pressure (mm Hg)	51 ± 3	43 ± 2**
Arterial blood glucose (mg%)	101.9 ± 5.0	96.3 ± 3.3
Arterial blood lactate (mg%)	10.5 ± 1.3	7.8 ± 1.2**
Arterial plasma free fatty acids (μ eq/ml)	1.2 ± 0.1	1.0 ± 0.1*
Rectal temperature (°C)	39.2 ± 0.2	39.0 ± 0.1

TABLE XVII BLOOD AND TEMPERATURE PARAMETERS PRIOR TO AND FOLLOWING THE PROLONGED EXPERIMENTS (50%L + 65%L GROUPS)[a]

[a] Values are $\bar{X}$ ± SEM
* Significant change with time ($p < .05$)
** Significant change with time ($p < .01$)

EXPERIMENT NUMBER	ASSIGNED GROUP	INITIAL TENSION KG	INITIAL TENSION G/G	FINAL TENSION KG	FINAL TENSION G/G	STIMULATED MUSCLE WEIGHT (G)
9	50%L	14.0	134	6.8	65	104.1
10	50%L	19.2	230	7.9	94	83.5
11	50%L	9.8	108	5.0	55	90.6
12	80%	10.0	136	7.8	106	73.7
13	50%	26.8	296	9.2	102	90.6
14	50%	17.4	288	8.4	139	60.5
15	65%	21.4	212	13.2	131	100.9
16	65%L	12.0	202	7.8	131	59.4
17	65%	16.0	365	10.2	233	43.9
18	65%	10.8	117	7.0	76	92.4
19	65%L	14.4	148	10.3	105	97.6
20	65%L	15.4	406	10.9	287	37.9
21	80%	19.2	210	15.2	166	91.6
22	80%	20.8	246	16.2	191	84.7
23	50%	18.8	259	8.3	114	72.7
24	50%L	13.6	161	7.4	88	84.3
26	50%L	19.2	284	9.6	142	67.6
27	80%	14.4	188	11.7	153	76.4
28	50%L	16.4	302	8.8	162	54.3
29	65%	20.4	200	13.3	130	102.1
30	80%	12.0	150	9.2	115	80.2
31	50%L	17.6	190	8.9	96	92.8
32	65%	20.4	200	13.3	130	102.1

CONT'D

EXPERIMENT NUMBER	ASSIGNED GROUP	INITIAL TENSION KG	INITIAL TENSION G/G	FINAL TENSION KG	FINAL TENSION G/G	STIMULATED MUSCLE WEIGHT (G)
33	65%L	12.4	288	8.0	186	43.0
34	65%L	17.6	233	10.6	140	75.5
35	50%	18.4	244	8.8	117	75.7
36	50%	25.6	277	13.6	147	92.4
37	50%	21.0	206	10.8	106	102.1
38	65%	21.2	252	12.8	152	84.2

TABLE XVIII DEVELOPED MUSCLE TENSION AT THE BEGINNING AND END OF STIMULATION, AND LEFT MUSCLE WEIGHT

PARAMETER NUMBER	PARAMETER
1	Tension at sample (% of original)
2	Tension at sample (kg)
3	Rate of tension decline (g/min)
4	Time from beginning of stimulation (min)
5	Rate of tension decline (%/min)
6	Tension at sample (g/g)
7	Rate of tension decline (g/g/min)
8	Lactate (A-V) difference (μM/g/min)
9	Water (A-V) difference (μM/g/min)
10	Glucose (A-V) difference (μM/g/min)
11	Free fatty acid (A-V) difference (neq/g/min)
12	Muscle $\dot{V}O_2$ (μl/g/min)
13	Muscle blood flow (ml/g/min)
14	Systolic pressure (mmHg)
15	Muscle temperature (°C)
16	Diastolic pressure (mmHg)
17	Respiratory Quotient
18	Venous blood pH
19	Venous blood pCO_2 (mmHg)
20	Arterial blood pO_2 (mmHg)
21	Venous blood pO_2 (mmHg)
22	O_2 (A-V) difference (mmHg)

TABLE XIX NUMBER ASSIGNMENT FOR PARAMETERS CONTAINED IN TABLE XX

	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	—												
2.	.51*	—											
3.	.43*	.62*	—										
4.	-.47*	-.58*	-.46*	—									
5.	.42*	.54*	.98*	-.49*	—								
6.	.62*	.55*	.32*	-.39*	.29	—							
7.	.47*	.59*	.99*	-.48*	.98*	.40*	—						
8.	-.37*	-.41*	-.35*	.63*	-.35*	-.37*	-.37*	—					
9.	.12	.27	.18	-.03	.15	.24	.18	-.09	—				
10.	-.23	-.24	-.09	.12	-.08	-.13	-.11	.18	.05	—			
11.	.13	.14	.17	-.09	.16	.17	.20	-.05	.31	-.23	—		
12.	.36*	.19	-.14	-.13	-.17	.13	-.17	-.16	.04	.01	-.24	—	
13.	.14	-.33*	-.35*	.27	-.34*	-.07	-.33*	.20	.09	-.03	.03	.53*	—
14.	.03	-.06	-.10	-.06	-.09	-.05	-.06	-.01	-.10	-.07	.16	.14	.33*
15.	-.07	.17	.10	-.16	.09	-.21	.06	-.07	.05	-.08	-.26	.21	.04
16.	.21	.22	.01	-.16	-.04	.16	-.02	-.13	-.10	-.09	-.09	.10	.02

	1	2	3	4	5	6	7	8	9	10	11	12	13
17.	.20	.23	.25	-.21	.25	.19	.23	-.23	.19	-.17	.09	-.32*	-.29
18.	-.27	-.30*	-.41	.29	-.42*	-.17	-.39*	.31*	-.07	-.07	.09	-.08	.34*
19.	.46*	.61*	.38*	-.44*	.33*	.25	.35*	-.43*	.01	-.24	.07	.15	-.30
20.	.03	.17	.12	-.14	.09	.15	.11	-.29	-.01	.08	-.29	.24	.02
21.	-.07	-.45*	-.20	.28	-.11	-.24	-.19	.26	-.14	-.01	-.06	-.26	.39*
22.	.08	.39*	.21	-.27	.14	.26	.20	-.38*	.06	.08	-.21	.37*	-.16

CONT'D

	14	15	16	17	18	19	20	21	22
14.	—								
15.	-.03	—							
16.	-.31*	.33	—						
17.	-.25	.02	.30	—					
18.	.03	.19	.04	-.02	—				
19.	-.18	.02	.45*	.37*	-.54*	—			
20.	-.08	.31	.28	.05	.05	.17	—		
21.	.08	.14	.19	.20	.21	-.27	.04	—	
22.	-.10	.23	.14	-.04	-.06	.30*	.83*	-.52*	—

TABLE XX CORRELATION MATRIX OF ARTERIOVENOUS AND TENSION-TIME PARAMETERS

*significant correlation ($p < .01$)

PARAMETER NUMBER	PARAMETER
1	Tension at sample (% of original)
2	Tension at sample (kg)
3	Rate of tension decline (g/min)
4	Time from beginning of stimulation
5	Rate of tension decline (%/min)
6	Tension at sample (g/g)
7	Rate of tension decline (g/g/min)
8	Right muscle weight (g)
9	Left muscle weight (g)
10	Difference in muscle weights (L minus R)(g)
11	Right muscle glycogen content (g%)
12	Left muscle glycogen content (g%)
13	Muscle glycogen L/R ratio
14	Difference in muscle glycogen content (L minus R)(g)
15	Left muscle lactate concentration (μM/g)
16	Left muscle ATP concentration (μM/g)
17	Left muscle CP concentration (μM/g)
18	Level-off tension (% of original)
19	R.Q. at level-off
20	Muscle $\dot{V}O_2$ at level-off (μl/g/min)
21	Muscle blood flow at level-off (ml/g/min)
22	Left muscle cytoplasmic $[NAD^+]/[NADH]$ ratio
23	Left muscle mitochondrial $[NAD^+]/[NADH]$ ratio
24	Left muscle temperature at sample (°C)
25	Systolic pressure at sample (mmHg)

CONT'D

PARAMETER NUMBER	PARAMETER
26	Diastolic pressure at sample (mmHg)
27	Glucose (A-V) difference at level-off (μM/g/min)
28	Lactate (A-V) difference at level-off (μM/g/min)
29	Free fatty acid (A-V) difference at level-off (neq/g/min)
30	Water (A-V) difference at level-off (μl/g/min)
31	% fast twitch fibres in level-off groups
32	% slow twitch fibres in level-off groups
33	Glucose (A-V) difference at 65% in level-off experiments (μM/g/min)
34	Lactate (A-V) difference at 65% in level-off experiments (μM/g/min)
35	Free fatty acid (A-V) difference at 65% in level-off experiments (neq/g/min)
36	Water (A-V) difference at 65% in level-off experiments (μl/g/min)
37	Muscle blood flow at 65% in level-off experiments (ml/g/min)
38	Muscle $\dot{V}O_2$ at 65% in level-off experiments (μl/g/min)
39	Arterial hematocrit at level-off (%)
40	Arterial pH at level-off
41	Arterial pO_2 at level-off (mmHg)
42	Venous pO_2 at level-off (mmHg)
43	O_2 (A-V) difference at level-off (mmHg)

TABLE XXI NUMBER ASSIGNMENT FOR PARAMETERS CONTAINED IN TABLE XXII

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.	—														
2.	.39	—													
3.	.37	.60*	—												
4.	-.44	-.66*	-.62*	—											
5.	.41	.53*	.97*	-.68*	—										
6.	.44	.52*	.37	-.29	.32	—									
7.	.38	.57*	.99*	-.63*	.97*	.40	—								
8.	.13	.66*	.22	-.37	.19	-.01	.18	—							
9.	-.06	.62*	.22	-.48	.18	-.49	.16	.96*	—						
10.	-.29	.30	.20	-.40	.23	-.25	.18	.21	.49	—					
11.	.33	.42	.31	-.08	.23	.22	.27	.41	.21	-.24	—				
12.	.52*	.57*	.45	-.42	.39	.32	.43	.38	.29	-.01	.79*	—			
13.	.15	.08	.10	-.42	.17	.03	.13	-.18	.10	.42	-.54*	.02	—		
14.	.03	.12	.05	.27	-.01	.05	.02	.32	.06	-.38	.81*	.29	-.87*	—	
15.	.40	.63	.79*	-.63*	.76*	.31	.78*	.20	.28	.35	.28	.51*	.21	-.04	—
16.	-.05	-.01	-.13	.21	-.15	.28	-.13	.01	-.26	-.25	.12	-.05	-.35	.24	-.34

CONT'D

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
17.	-.62*	-.23	-.38	.47	-.44	-.32	-.39	.03	.10	.01	-.02	-.13	-.25	.10	-.48
18.	1.00*	.10	-.10	.07	-.10	.50	-.10	.17	-.28	-.50	.19	.37	-.04	.05	-.52
19.	.21	.36	-.05	.09	-.05	.48	-.05	.12	.07	.08	.54	.48	-.13	.42	-.50
20.	.54	-.42	-.12	.37	-.12	-.05	-.12	-.61	-.48	-.61	-.11	-.08	.14	-.09	.00
21.	.51	-.43	-.46	.24	-.46	-.02	-.46	-.22	-.34	-.34	-.16	-.11	.09	-.15	-.22
22.	-.17	-.14	-.21	.36	-.25	.24	-.22	-.10	-.30	-.30	.16	-.21	-.51*	.45	-.49*
23.	.09	.00	-.06	.24	-.13	-.23	-.08	.39	.22	-.36	.36	.22	-.34	.41	-.24
24.	-.08	-.06	.24	-.14	.29	-.28	.25	.07	.12	.21	-.10	.06	.27	-.23	.19
25.	.02	-.09	-.22	-.08	-.15	-.18	-.22	-.19	.04	.19	-.22	-.12	.16	-.22	-.05
26.	.36	.41	.18	-.30	.16	.19	.15	.55*	.32	-.11	.51*	.40	-.33	.42	.09
27.	-.10	-.21	.08	-.20	.08	.10	.08	-.66	-.46	-.14	-.26	-.42	.22	-.12	.24
28.	.06	.30	-.04	.03	-.04	.09	-.04	-.19	.05	.12	.10	.44	.23	-.10	-.01
29.	-.08	.17	-.04	.20	-.04	.16	-.04	.28	.02	-.19	.02	.04	-.18	.01	-.02
30.	.57	-.08	-.13	-.28	-.13	.57	-.13	-.34	-.63	-.68	-.17	.24	.50	-.34	-.10
31.	.04	.04	.00	.35	.00	.64	.00	-.34	-.39	-.32	.66	.13	-.46	.78	-.34
32.	-.04	-.04	.00	-.35	.00	-.64	.00	.34	.39	.32	-.66	-.13	.46	-.78	.34

CONT'D

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
33.	-.06	-.72	-.06	-.17	-.06	-.29	-.06	-.76	-.54	-.22	-.67	-.43	.83*	-.68	.54
34.	.82*	-.33	-.14	-.51	-.14	.05	-.14	-.27	-.34	-.45	-.46	-.29	.41	-.47	-.02
35.	-.11	-.69	-.07	-.36	-.07	-.61	-.07	.22	.23	-.02	-.28	-.61	-.78	.04	.11
36.	.22	.57	-.48	-.03	-.48	.16	-.48	.70	.47	-.20	.64	.45	-.55	.62	-.82*
37.	.57	-.51	-.46	-.16	-.46	-.08	-.46	-.21	-.32	-.38	-.44	-.22	.47	-.47	-.08
38.	.21	-.74	-.45	-.64	-.45	-.59	-.45	-.53	-.09	-.22	-.72	-.51	.78*	-.69	.20
39.	.17	.06	.62	.52	.62	.48	.62	-.12	-.34	-.18	.55	.28	-.42	.53	-.02
40.	.22	.60	.07	-.42	.07	.24	.07	.61	.38	-.15	-.02	.36	.23	-.22	-.19
41.	.09	-.29	-.14	.10	-.14	-.13	-.14	.21	.01	-.53	.32	-.19	-.44	.50	-.29
42.	.32	-.16	-.66	..39	-.66	.06	-.66	.11	-.06	.02	.30	.00	-.37	.37	-.64
43.	-.12	-.17	.30	-.15	.30	-.17	.30	.14	.05	-.53	.12	-.19	-.20	.25	.13

CONT'D

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
16.	—														
17.	.26	—													
18.	.30	-.22	—												
19.	.56	-.08	.21	—											
20.	-.31	-.01	.46	-.13	—										
21.	-.25	.02	.50	-.23	.67	—									
22.	.65*	.42	.27	.80*	-.29	-.05	—								
23.	.23	.25	.64	.01	.02	-.05	.30	—							
24.	.26	-.05	.12	-.29	-.12	.25	-.05	.45	—						
25.	-.35	-.24	-.42	-.50	.16	.40	-.16	-.25	-.02	—					
26.	.43	-.14	.49	.40	-.22	.14	.25	.17	.35	-.17	—				
27.	-.23	-.38	-.06	-.01	.33	-.16	.02	.06	-.55	.09	-.57	—			
28.	.32	.72	-.07	.15	.21	.07	.14	-.14	.45	.00	-.19	.12	—		
29.	-.35	-.51	-.08	.09	-.21	-.06	-.31	-.24	-.57	.10	-.02	-.22	-.46	—	
30.	.23	-.50	.57	.44	.30	.39	.29	-.09	-.04	-.21	.18	.10	.12	.25	—
31.	.51	-.10	.04	.65	.11	-.07	.84*	-.18	.09	-.32	.48	.54	.22	-.40	.30

CONT'D

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
32.	-.51	.10	-.04	-.65	-.11	.07	-.84*	.18	-.09	.32	-.48	-.54	-.22	.40	-.30
33.	-.52	.06	-.06	-.35	.78*	.62	-.31	-.53	.47	.55	-.35	.25	.35	-.30	.45
34.	-.26	-.26	.82*	-.55	.68	.54	-.15	.59	.08	.10	-.14	.39	-.08	-.31	.42
35.	-.20	-.66	-.11	-.05	-.66	-.44	-.18	.11	-.37	.12	.19	-.36	-.96*	.31	-.53
36.	.29	-.09	.22	.48	-.40	-.14	.45	.66	-.06	-.08	.47	-.23	-.28	.43	.07
37.	-.31	-.04	.57	-.42	.66	.98*	-.04	.01	.48	.53	.23	-.16	.02	-.12	.68
38.	-.56	-.02	.21	-.38	.73	.68	-.26	.19	.41	.72	-.28	.12	.05	-.28	.38
39.	.28	-.41	.17	.59	.05	-.31	.31	-.45	-.47	-.72	.41	.09	-.19	.06	.11
40.	-.05	-.01	.22	.00	-.44	-.26	-.08	.95	-.56	-.29	-.18	-.08	-.04	.42	.18
41.	-.15	-.48	.09	.07	-.10	.01	.33	.73	.03	.00	.49	-.07	-.75*	.07	-.11
42.	.10	-.07	.32	.35	.23	.64	.52	-.09	.20	.31	.55	-.37	-.13	.12	.25
43.	-.21	-.43	-.12	-.16	-.25	-.41	.01	.57	-.10	-.20	.13	.18	-.66	-.01	-.27

CONT'D

	31	32	33	34	35	36	37	38	39	40	41	42	43
31.	—												
32.	-1.00*	—											
33.	-.02	.02	—										
34.	-.09	.09	.30	—									
35.	-.45	.45	-.67	-.07	—								
36.	.18	-.18	-.73	-.18	.27	—							
37.	-.10	.10	.62	.59	-.22	-.16	—						
38.	-.30	.30	.78*	.54	.19	-.38	.73	—					
39.	.49	-.49	-.24	-.40	.16	-.10	-.60	-.55	—				
40.	-.44	.44	-.53	.18	-.06	.46	-.17	-.32	-.25	—			
41.	.32	-.32	-.31	.06	.76	.49	.07	.02	.19	-.16	—		
42.	.19	-.19	.09	-.08	.14	.38	.55	.28	-.02	-.32	.31	—	
43.	.20	-.20	-.36	.11	.60	.25	-.27	-.15	.20	.05	.79*	-.35	—

TABLE XXII CORRELATION MATRIX OF MUSCLE CONCENTRATION AND TENSION-TIME PARAMETERS

*significant correlation ($p < .01$)

APPENDIX F

PLATE V MICROGRAPHS OF SERIAL SECTIONS FROM RIGHT CONTROL GASTROCNEMIUS (MEDIAL HEAD) (100X magnification)

A. Myosin ATPase

B. NADH DIAPHORASE

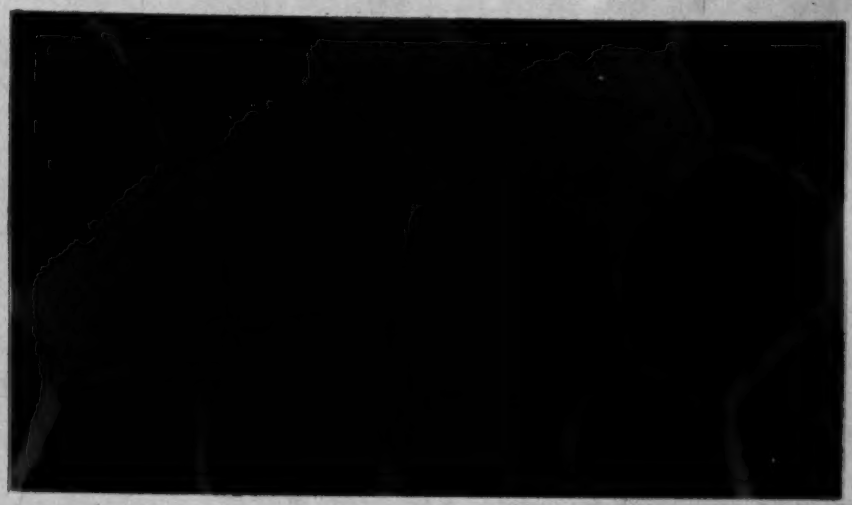
C. PERIODIC ACID-SCHIFF (PAS)

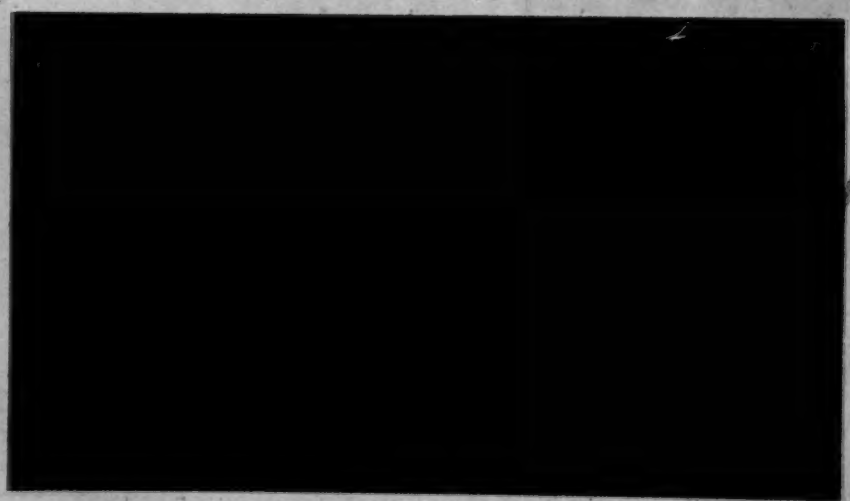
PLATE VI MICROGRAPHS OF SERIAL SECTIONS FROM RIGHT CONTROL GASTROCNEMIUS (MEDIAL HEAD) (250X magnification)

A. MYOSIN ATPase

B. NADH DIAPHORASE

C. PERIODIC ACID-SCHIFF (PAS)





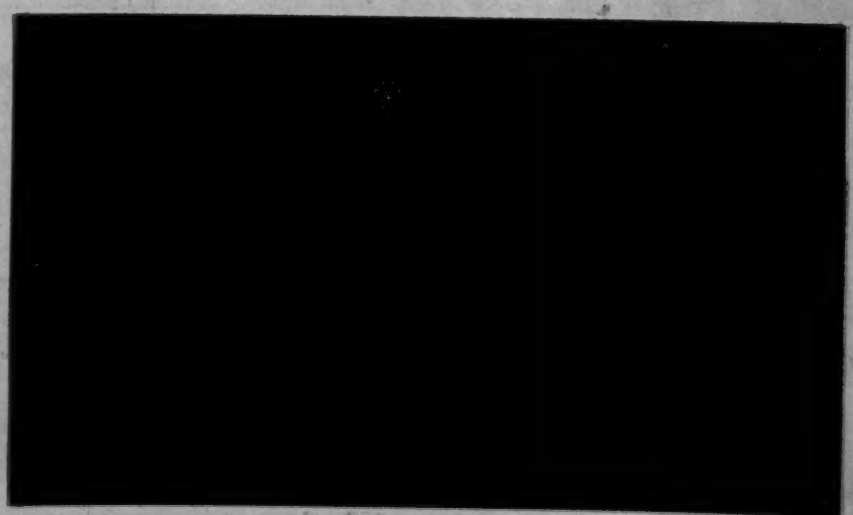


PLATE VII MICROGRAPHS OF SERIAL SECTIONS FROM STIMULATED MUSCLE OF 80% GROUP (100X magnification)

A. MYOSIN ATPase

B. PERIODIC ACID-SCHIFF (PAS)

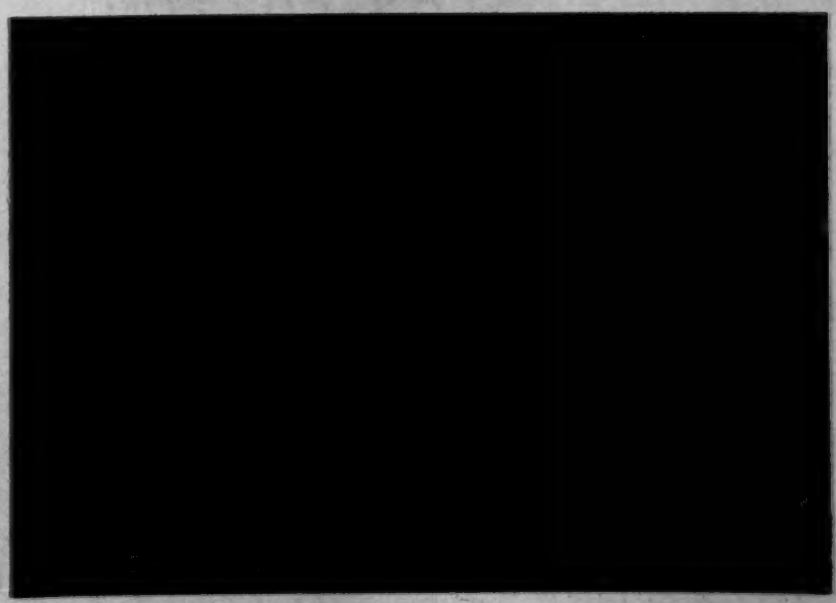


PLATE VIII MICROGRAPHS OF SERIAL SECTIONS FROM STIMULATED MUSCLE OF 65% GROUP (100X magnification)

A. MYOSIN ATPase

B. PERIODIC ACID-SCHIFF (PAS)

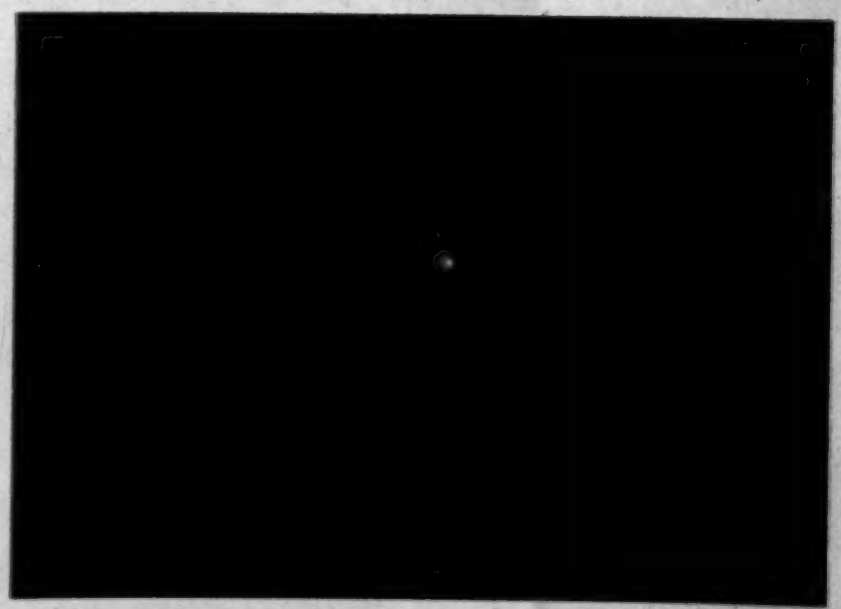
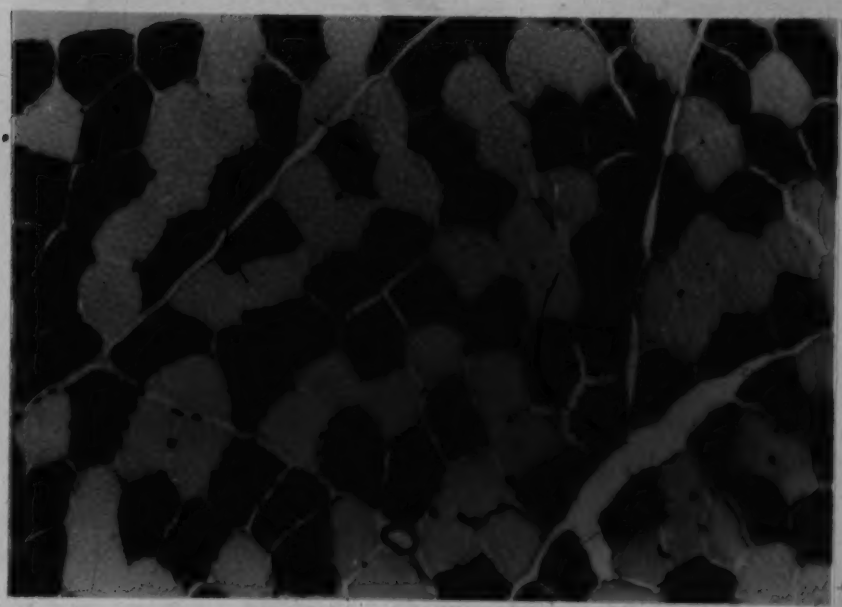


PLATE IX MICROGRAPHS OF SERIAL SECTIONS FROM STIMULATED MUSCLE OF 50% GROUP (100X magnification)

A. MYOSIN ATPase

B. PERIODIC ACID-SCHIFF (PAS)



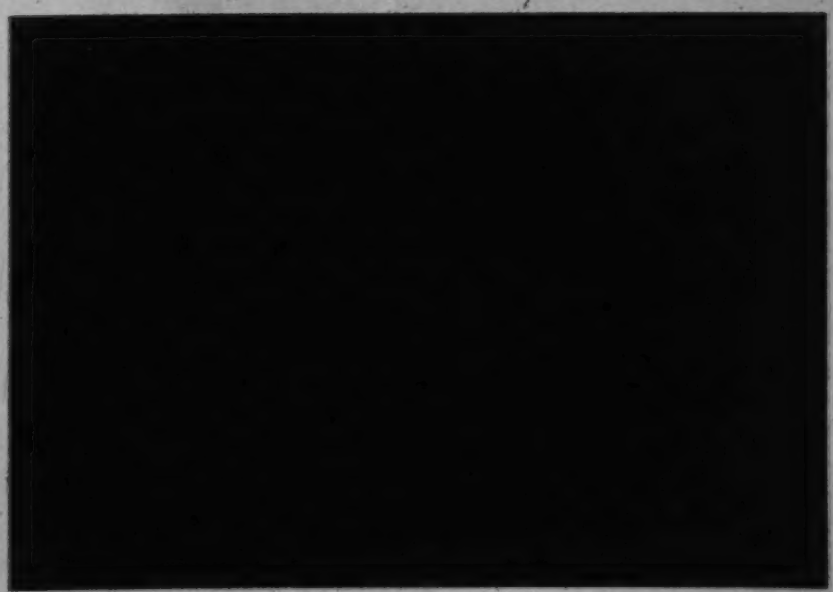


PLATE X MICROGRAPHS OF SERIAL SECTIONS FROM STIMULATED MUSCLE OF 65%L GROUP (100X magnification)

A. MYOSIN ATPase

B. PERIODIC ACID-SCHIFF (PAS)

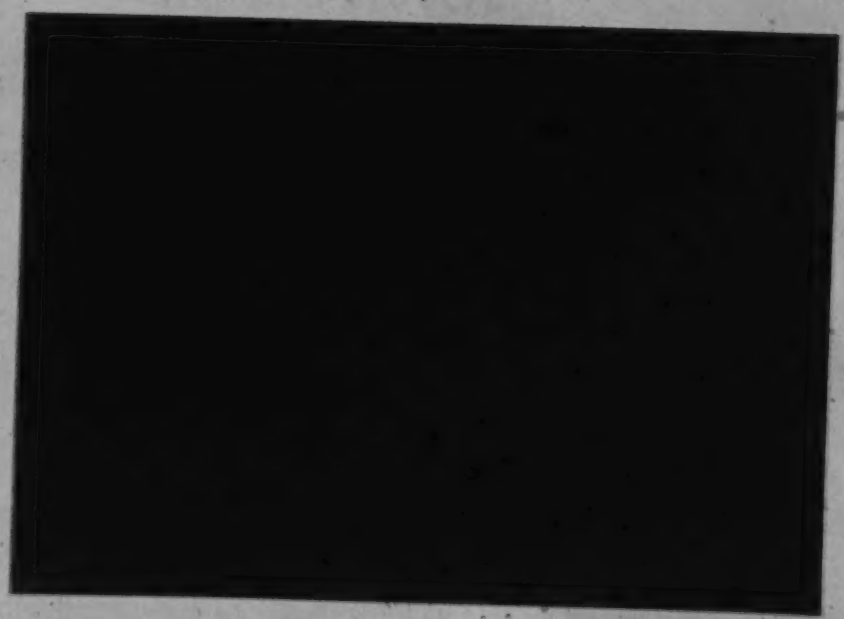
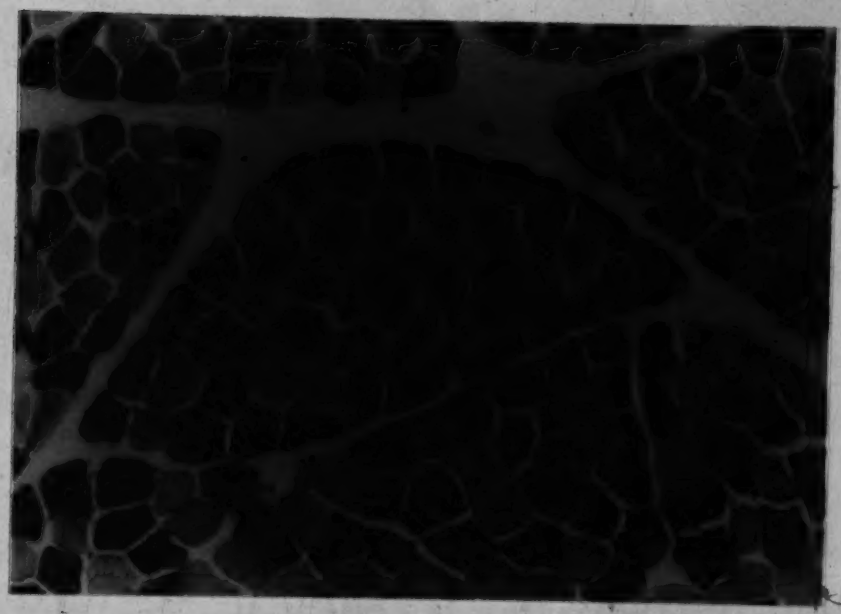
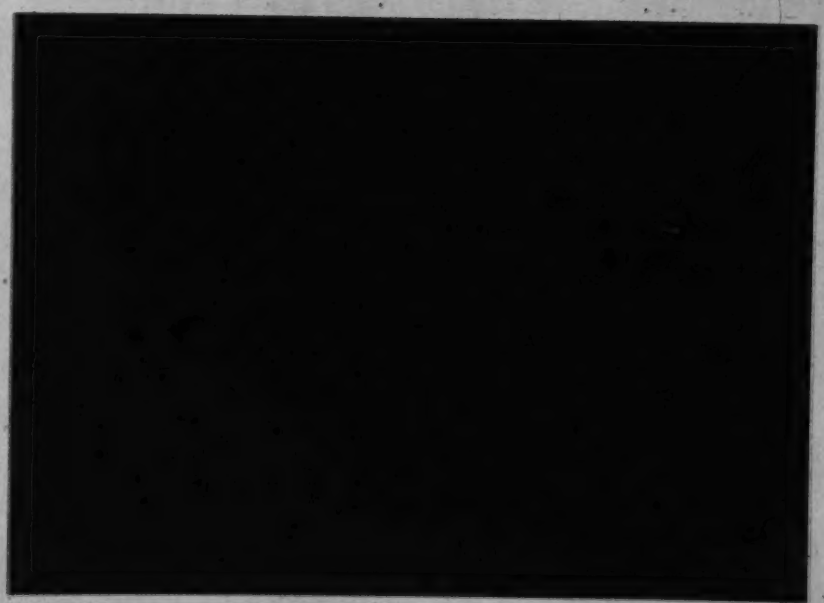


PLATE XI MICROGRAPHS OF SERIAL SECTIONS FROM STIMULATED MUSCLE OF 50%L GROUP (100X magnification)

A. MYOSIN ATPase

B. PERIODIC ACID-SCHIFF (PAS)